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INFLUENCE OF WILD AND CULTIVATED PLANTS ON THE MULTIPLICATION, SURVIVAL AND SPREAD OF CEREAL FOOT-ROTTING FUNGI IN THE SOIL[1]

By G. W. Padwick[2]

Abstract

Using the severity of infection of wheat seedlings as a measure of soil infestation, it is shown that susceptible grasses such as *Agropyron tenerum*, *A. cristatum*, *A. repens* and *Bromus inermis* encourage the multiplication and survival of inoculum of the take-all fungus *Ophiobolus graminis* in both sterilized and unsterilized soil. The same grasses also aided the survival of *Helminthosporium sativum* in sterilized soil. In these experiments, however, only one, namely *B. inermis*, appeared to favor the survival of *Fusarium graminearum*.

The fungus *O. graminis*, which failed to spread laterally to any appreciable extent in bare, unsterilized black loam soil of the Edmonton district of Alberta, was able to do so when such soil was occupied by living, susceptible plants.

Introduction

The present study was undertaken primarily with the object of ascertaining in what ways the growth of higher plants may influence the subterranean behavior of the fungi causing foot rots of wheat. Three foot-rotting organisms were selected for study, namely *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P.K.B. and *Fusarium graminearum* Schwabe. At the beginning of the investigation it was realized that several effects of the growth of higher plants on such fungi may be possible. Of these, five are suggested as having an important influence.

(i) By serving as selective media for the parasites, to the exclusion of the saprophytes which normally by their competitive action help to keep the parasites in check, higher plants of susceptible species may permit an abundance of inoculum to be built up.

(ii) Susceptible plants may be an important means by which pathogenic organisms persist in field soils.

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(iii) The pathogenes may spread through the soil more rapidly on, or in, the roots of susceptible plants than in bare soil where the spread is checked by competing organisms.

(iv) Higher plants, either living or dead, may be the chief means of overwintering of the pathogenes in the soil.

(v) The living underground parts of plants may secrete substances capable of stimulating or of inhibiting the growth of organisms in the soil, and the presence of dead tissue of such plants may serve the same purpose.

The first three aspects of the problem have been studied experimentally.

Review of Literature

A disease somewhat analogous to cereal foot and root rots is the cotton root-rot disease, which causes serious losses in the United States of America. Taubenhaus and Killough (31) in 1923 stated their belief that clean culture involving the destruction of the winter carriers of the causal fungus, *Phymatotrichum omnivorum*, was of value in helping to control the cotton root-rot disease, while rotations involving only partial freedom from susceptible hosts were not effective. Taubenhaus, Dana and Wolff (30) in 1929 concluded that it was impossible to control cotton root rot without destroying susceptible perennial weeds. McNamara and Hooton (18) found a one-year fallow insufficient, a two-year fallow or a one-year fallow in combination with a rotation system being necessary for control of cotton root rot.

In the case of wheat, the effect of rotations on root-rot development has been studied by a great many workers. This work has recently been so thoroughly reviewed by Broadfoot (3) that it is unnecessary to go into details here. Special attention however may be called to the names of Henry (11), Sewell and Melchers (28), Greaney and Bailey (10), Russell (23, 24) and Sanford (26). Similarly, the literature on the host range of the three fungi has been reviewed by Padwick and Henry (20).

All the organisms studied here are able to carry on a saprophytic existence in some soils, but the extent to which they do so under natural conditions is not easily determined.

Kirby (16) found that soil infested with *Ophiobolus graminis*, when screened and kept indoors for eight months, lost its ability to cause take-all of wheat, while bits of straw containing perithecia were able to cause severe infection when similarly treated. Davis (5) and Russell (22), however, have found this fungus to persist in bare soil for considerable periods. Russell for instance (25) found that *O. graminis* can remain viable in bare soil for at least two years and then infect wheat seeded in the soil, but that there was a marked decrease with time in the aggressiveness shown by the fungus.

Henry (12) found strong inhibition of *H. sativum* produced by the addition to sterilized soil of small quantities of unsterilized black loam soil at the same time that inoculum of the pathogene was added, and suggested that under natural conditions micro-organisms comprising the natural soil flora have a

marked suppressive action on foot-rotting organisms. The same writer (13) found that spores of *H. sativum* occur rarely if ever in field soils, owing to the inhibitive influence of other micro-organisms. He later (14) found in studies with *O. graminis* that this inhibitive action of the normal soil flora varies with temperature. Attacking the problem in a different way, Sanford and Broadfoot (27) and Broadfoot (2) studied the mode of action of other soil inhabiting micro-organisms on the virulence of *O. graminis*, finding numerous inhibitors and a few compatible organisms.

Garrett (9) utilized the rate at which hyphae of *Ophiobolus graminis* grew externally along seminal roots of wheat as an indication of the suitability of the environmental conditions of the soil for infection of wheat. Soil saprophytes were found to impede the progress of the hyphae.

The difficulty of isolating *Ophiobolus graminis* from the soil and from infected plants, its weak saprophytic growth, and the inconspicuous nature of the symptoms of the disease it causes when in the early stage, have made a knowledge of the distribution and progress of the organism in the soil under field conditions difficult to obtain. Russell (23) was unable to demonstrate the spread of take-all from inoculated seedlings in the centre of eight-inch crocks to seedlings sown closely around them, the neighboring plants reaching maturity without showing symptoms. Fellows (7) has found the organism present to a depth of at least ten inches in infested soils in Kansas, but it is possible that the organism may have been carried to this depth by deep plowing. It seems to be the popular belief that the circular patches of diseased wheat plants in fields are the result of the radial growth of *O. graminis* from the centre of infection. Similar circular spots in *A. repens* have been observed (20) to be very marked in a field which remained uncultivated for several years and was supporting a thick stand of this weed.

Influence of Weeds and Grasses on the Multiplication and Survival of Foot-rotting Organisms in the Soil

Experimental Methods

Five species of higher plants of economic importance in the prairie provinces of Canada were selected in order to determine their effect on the survival of *O. graminis*, *H. sativum* and *F. graminearum* in the soil and their role in increasing the amount of inoculum for infecting wheat. Four of the plants studied were gramineous species, namely *Agropyron tenerum* (slender wheat grass) and *A. cristatum* (crested wheat grass), which are cultivated as forage crops in western Canada; *A. repens* (couch grass), a common weed in many parts, and *Bromus inermis* (brome grass), which is grown extensively for forage purposes. All these grasses are perennials. The fifth species was a dicotyledonous plant, *Neslia paniculata* (ball mustard), an annual weed.

The method adopted was to apply inoculum of the various organisms to pots of soil, together with seed of the species to be studied, and after a period of growth varying from five to eight weeks to seed the pots to wheat (*Triticum*

vulgare), which served as an indicator of the survival of the fungus in the soil and of its ability to reinfect wheat in planted soil as compared with controls in unplanted soil. Some experiments were run concurrently, using the same controls; others were run separately and separate controls had to be used. In certain instances, owing to unsuitable temperatures obtained in the greenhouse while the experiments were being conducted, some series, together with their controls, had to be repeated in their entirety.

The inoculum was prepared by growing the organisms in Erlenmeyer flasks containing 50 gm. of black loam soil which was moistened with 28 cc. of tap water and sterilized by autoclaving. The fungus was allowed to grow in the flasks for 17 days at room temperature. Six-inch pots were two-thirds filled with black loam soil and half of the pots were then sterilized in the autoclave for four hours. The entire contents of a flask was then added to each pot. Seeds of the plant species to be studied were then sown in the pots and covered with soil. Ten replicates of each treatment were seeded, so that every species under investigation was grown in ten pots with each of the three fungi in sterilized soil, and ten pots in unsterilized soil. Other pots were filled and had fungi added in a similar manner, but were not seeded, and these were used for comparison with the seeded pots. All the pots were then placed in the greenhouse at approximately 25° C., and the plants were allowed to grow for five to eight weeks. The top growth of the plants was then cut off and each pot was seeded with 25 seeds of Marquis wheat, which had previously been treated with hot water in order to kill as far as possible any pathogenic fungi which might be present in or on the seeds. After three weeks the wheat seedlings were removed and were assigned infection ratings from 0 to 5 according to the degree of rotting of stems and roots. These were averaged for each pot and series of pots and expressed as percentages of the greatest possible degree of rotting.

Experimental Results

Considering the three organisms separately, (Table I) it is seen that in the case of *Ophiobolus graminis* there was in each instance a very marked and significant increase in the degree of infection of wheat following grasses in both sterilized and unsterilized soil. In fact, in the absence of weeds, there was no trace of infection when unsterilized soil was used. It is also clear that *Neslia paniculata* effected no significant difference in the amount of inoculum as measured by infection of wheat.

With *Helminthosporium sativum* the most striking results were found on sterilized soil, where, following all the grasses, with the exception perhaps of *Bromus inermis*, there was a very marked increase of infection. On unsterilized soil the grasses effected only a small increase, while *Neslia paniculata* effected none.

There was no significant increase of *Fusarium graminearum* foot rot as a result of the growth of species of *Agropyron*, nor was any increase caused by growth of *Neslia paniculata*. Wheat after *Bromus inermis* in unsterilized soil, however, showed a marked increase of *Fusarium* foot rot. There was

TABLE I

INFECTION OF WHEAT WITH *Ophiobolus graminis*, *Helminthosporium sativum* AND *Fusarium graminearum* FOLLOWING CERTAIN WEEDS AND GRASSES

Organism	Soil	Presence of grass or weed	*Agropyron tenerum*		*Agropyron cristatum*		*Agropyron repens*		*Bromus inermis*		*Neslia paniculata*	
			Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]
Ophiobolus graminis	Unsterilized	Absent	0		0		0		0		0	
	Unsterilized	Present	11.3	+ +	1.8	+	5.2	+ +	9.9	+ +	0	—
	Sterilized	Absent	0.4		0.4		0.4		0.4		0	
	Sterilized	Present	13.0	+ +	5.8	+ +	6.7	+ +	15.9	+ +	0	—
Helminthosporium sativum	Unsterilized	Absent	9.4		9.4		9.4		5.6		5.0	
	Unsterilized	Present	14.5	0	15.3	0	15.4	+	19.1	+ +	4.9	0
	Sterilized	Absent	3.3		3.3		3.3		31.8		30.7	
	Sterilized	Present	14.7	+ +	9.8	+ +	9.6	+ +	51.0	+ +	30.3	0
Fusarium graminearum	Unsterilized	Absent	17.5		17.5		17.5		3.6		7.2	
	Unsterilized	Present	23.0	0	22.2	0	20.0	0	27.1	+ +	7.8	0
	Sterilized	Absent	30.6		30.6		30.6		44.9		31.4	
	Sterilized	Present	26.1	0	20.2	0	32.5	0	41.1	0	21.4	0

[1] *Probability refers to the odds according to the method of Student. Odds below 30 : 1 are considered to mean that the difference is not significant (0); odds of 30 : 1 to 200 : 1 mean a significant difference (+); and above 200 : 1, very significant (+ +).*

no increase of infection in sterilized soil, but in view of the fact that in bare sterilized soil the infection reached 44.9%, much increase would perhaps not be expected.

The results in general show a close agreement with what was to be expected from results of experiments previously reported (20). In those experiments it was found that all the species of *Agropyron* studied were severely damaged by *O. graminis*, as also was *Bromus inermis*. The present results show that, in bare unsterilized soil, the organism, under the conditions existing, seemed to have become incapable of attacking wheat, while in soil planted to *A. tenerum*, *A. cristatum*, *A. repens* and *B. inermis* considerable infection of the wheat occurred. In all cases a marked increase occurred in the degree of infection of wheat with *O. graminis* in unsterilized soil after susceptible grasses, indicating a tendency for the organism to accumulate in soil planted to these grasses.

In the previous experiment all the four grasses studied were found susceptible to *H. sativum*, and all have served in the present experiment to increase the amount of inoculum of this organism in sterilized soil. The amounts of increase in unsterilized soil were not very significant. It was previously found that none of the four grasses appeared to be damaged by *H. sativum* in unsterilized soil to the same extent as they were by *O. graminis*, and for this reason it was only to be expected that the grasses would play a somewhat smaller role in carrying over the organism and serving as a source of inoculum for the succeeding wheat. *B. inermis* was the only grass of the four studied which in the previous experiments was infected and damaged by *F. graminearum* in unsterilized soil, and it is the only grass which in these experiments served to increase the amount of inoculum of this organism in unsterilized soil. In no instance did *Neslia paniculata* serve to aid in the survival or increase of any wheat foot-rotting pathogenes in the soil.

These results support the view previously expressed that the mere fact that a plant is susceptible to attack by wheat foot-rotting pathogenes under the unusual conditions of experimental inoculation, especially in sterilized soil, does not give an adequate indication of the role which it may play in the problem of foot rot in wheat. It is necessary to obtain an indication of the relative amount of damage done to these susceptible plants, in unsterilized as well as sterilized soil, before a reliable estimate of their importance in the problem may be made.

The Effect of Grasses on the Horizontal Spreading of Foot-rotting Fungi in Soil

Experimental Methods

Improved technique, developed by Mr. F. R. Davies in this laboratory, in isolating *Ophiobolus graminis* from plant tissues has made possible a study of the conditions under which this organism survives and spreads. Extensive experiments were outlined to determine whether this organism, and also

Fusarium graminearum, are able to spread progressively through unsterilized soil, both when bare and when planted to various grasses and to wheat.

Owing to the extensiveness of the experiment and the large amount of isolation work involved, the experiments were conducted with the two organisms at different times, first with *F. graminearum* (in the summer of 1932) and then with *O. graminis* (in the winter of 1932-33). There were, however, no essential differences in the methods adopted. On June 28, 15 flat wooden boxes, 25 × 16 × $3\frac{1}{2}$ in., were filled to a depth of $2\frac{1}{2}$ in. with unsterilized black loam soil, obtained from land kept bare for five years. Three boxes were seeded with wheat, three with *Agropyron tenerum* and three with *A. repens*. About 200 seeds were sown in each box. The remaining six boxes were not seeded. All were placed in the greenhouse at a soil temperature of about 20° C. and kept watered. On July 29, after the plants had become well established, a narrow trench was dug to the full depth of the soil, two inches from one end and across the full width of each box. In this was placed inoculum of *F. graminearum*, prepared by growing the fungus for 17 days in Erlenmeyer flasks each containing 45 gm. of moist sterilized soil plus 5 gm. of cornmeal. The contents of two flasks were used for each box. Three of the unplanted boxes were treated in the same manner, while in the trenches of the remaining three was placed a similar quantity of sterilized soil and cornmeal which, however, had no fungus growing on it. Thus it was possible to compare the spread of the organism in the presence of the two grasses, in the presence of wheat, and in unplanted soil. The purpose of the uninoculated boxes was to check against the natural occurrence of the fungus in the soil, which would at once render the results unreliable. On September 7, after removal of the top growth, Marquis wheat, which had been treated with hot water to reduce the seed-borne foot-rotting fungi to a minimum, was seeded in rows across the box along the original trench, and in rows parallel to it and two inches apart. Twelve rows were seeded in all. Twenty seeds were placed in each row, a small hole being made for each seed with a wooden meat skewer, a new skewer being used for each row. On September 27, the wheat plants from one box under each treatment were dug up and measured, the degree of infection of each plant was recorded, and the underground parts of plants in all rows showing any trace of foot rot were removed. These underground parts were surface sterilized by dipping for $1\frac{1}{2}$ min. in mercuric chloride (1 gm. in a litre) and washing in 75% alcohol. The second group of boxes was harvested and treated similarly on October 1; and the third replicate on October 6.

A few slight modifications were made for *O. graminis*. As stated previously, the experiment was conducted during the winter, being commenced November 1. Inoculum of *O. graminis* (strain 108, obtained from *Agropyron repens*) was added November 29. In order to minimize washing of inoculum over the soil in the boxes, the boxes were tilted slightly, so that if the organism were carried at all by the water it would be in the opposite direction to that in which the rate of spread was to be determined. Only ten rows of wheat

TABLE II

RESULTS OF EXPERIMENTS ON THE SPREAD OF *Ophiobolus graminis* IN PLANTED AND UNPLANTED SOILS

Soil treatment	Distance from original place of application of inoculum, in.	Average length of wheat plants, cm.	Average degree of infection of wheat plants, %	Results of re-isolation trials of *O. graminis*
Soil left bare. Soil + cornmeal with no organism was placed at one end of the box. Included to check against natural occurrence of *O. graminis* in the soil.	0	26.0	0	−
	2	27.3	0	−
	4	23.2	0	−
	6	23.4	0	−
	8	23.8	0	−
	10	24.3	0	−
	12	21.8	0	−
	14	23.0	0	−
Soil left bare. *O. graminis* placed at one end of the box.	0	23.5	14.4	+
	2	24.1	1.1	+
	4	24.0	0	−
	6	23.8	0	−
	8	25.4	0	−
	10	23.2	0	−
	12	22.1	0	−
	14	23.7	0	−
Soil seeded to wheat. *O. graminis* placed at one end of the box.	0	28.8	14.1	+
	2	24.0	29.3	+
	4	20.0	29.4	+
	6	19.3	15.4	+
	8	22.0	7.7	+
	10	21.1	0.6	−
	12	21.5	0	−
	14	21.6	0	−
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of the box.	0	25.6	9.7	+
	2	21.6	24.7	+
	4	21.2	10.7	+
	6	18.8	8.8	+
	8	19.1	7.2	+
	10	17.9	0.7	+
	12	18.9	0.6	+
	14	18.8	0	−
Soil seeded to *A. repens*. *O. graminis* placed at one end of the box.	0	26.3	8.6	+
	2	26.8	18.9	+
	4	21.2	13.2	+
	6	20.8	1.0	−
	8	20.2	0	−
	10	21.0	0	−
	12	20.6	0	−
	14	17.8	0	−

were seeded, on January 20, 52 days after adding the inoculum to the end of the box. All three replicates were harvested on February 14. In sterilizing for re-isolation, silver nitrate was used instead of mercuric chloride. Small portions of roots were dipped in 1% silver nitrate solution for one minute, and the silver nitrate was then precipitated with concentrated sodium chloride solution, after which the portions were plated on potato-dextrose agar in Petri dishes.

TABLE III

RESULTS OF EXPERIMENTS ON THE SPREAD OF *Fusarium graminearum* IN PLANTED AND UNPLANTED SOILS

Soil treatment	Distance from original place of application of inoculum, in.	Average length of wheat plants, cm.	Average degree of infection of wheat plants, %	Results of re-isolation trials of *F. graminearum*
Soil left bare. Soil + cornmeal with no organism placed at one end of box. Included to check against natural occurrence of *F. graminearum* in the soil.	0	32.0	1.1	−
	2	26.2	3.6	−
	4	28.3	1.1	−
	6	25.5	1.0	−
	8	24.2	4.8	−
	10	24.7	4.4	−
	12	26.6	2.3	−
	14	26.9	3.4	−
	16	27.2	1.1	−
	18	26.6	2.5	−
Soil left bare. *F. graminearum* placed at one end of box.	0	27.5	11.9	+
	2	28.5	3.8	−
	4	27.9	12.6	−
	6	27.6	2.4	−
	8	27.1	1.3	+
	10	27.4	5.0	−
	12	22.6	1.0	−
	14	27.2	6.3	+
	16	22.6	3.2	+
	18	27.1	1.1	−
Soil seeded to wheat. *F. graminearum* placed at one end of box.	0	27.9	24.8	+
	2	27.6	16.9	+
	4	25.1	24.1	−
	6	23.0	17.4	−
	8	22.2	19.0	−
	10	20.8	22.9	−
	12	19.0	19.4	−
	14	22.3	12.1	+
	16	22.2	16.6	−
	18	21.3	16.7	−
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of box.	0	26.7	27.9	+
	2	26.5	21.2	+
	4	25.8	21.4	+
	6	23.4	26.1	−
	8	22.6	16.7	−
	10	24.1	12.9	+
	12	24.1	11.1	+
	14	24.0	10.9	−
	16	22.5	17.2	−
	18	22.9	18.5	−
Soil seeded to *A. repens*. *F. graminearum* placed at one end of box.	0	26.6	26.9	+
	2	27.2	27.5	+
	4	25.2	23.2	−
	6	24.9	30.8	+
	8	23.1	32.4	−
	10	24.4	27.4	−
	12	24.0	32.1	−
	14	23.5	23.9	−
	16	24.1	31.0	−
	18	22.9	17.2	+

Experimental Results

The average length and the average degree of infection in per cent of each row of wheat plants, together with the results of the isolations, are given in detail in Tables V and VI. They are also summarized in Tables II and III, in which the plant lengths and degrees of infection given are the averages for the plants of all three replicates grouped together. The distances of spread of *O. graminis* in each box under the various treatments are shown in Table IV.

TABLE IV

DISTANCES WHICH *O. graminis* SPREAD IN TWO MONTHS IN BARE SOIL AND IN SOIL SEEDED TO *A. tenerum*, *A. repens*, AND WHEAT

Soil treatment	Distance which *O. graminis* spread in the soil (to nearest 2 in.)		
	Series 1, in.	Series 2, in.	Series 3, in.
Bare soil	0	2	0
Wheat	6	8	8
A. tenerum	4	12	8
A. repens	4	4	4

The results with *O. graminis* are striking and significant. Not only are there appreciable differences in the distances the organism spread under different conditions, but also there is, in most cases, quite close agreement between the replicates of each series. Table V shows that the fungus spread in only one of the three boxes of unplanted soil, and there it only spread about two inches and caused a small amount of infection of wheat. In boxes growing wheat it spread about six inches in one box and eight inches in the other two; under *A. tenerum* it spread from four to twelve inches; and in all boxes of *A. repens* it spread four inches. The organism was in no instance isolated from wheat plants from the uninfested control boxes.

Results with *F. graminearum* were irregular and less conclusive. While considerable care was taken in watering the boxes, they were not sloped and some washing of soil no doubt occurred. The effects of this were accentuated by the presence of spores in the inoculum. That these spores were carried for considerable distances is suggested by the fact that single infected plants were found isolated from other infected plants by as much as fourteen inches in one instance. The *F. graminearum* causing this infection evidently came from the original inoculum, since in no instance was the organism isolated from uninfested soil. It is unfortunate that the wheat plants for isolation were allowed to grow for so long a period before they were removed from the soil. Especially in the last two series harvested there was so heavy an infection with *Helminthosporium sativum* (indicated by H in the isolation column) occurring naturally in the soil that the degrees of infection recorded in the columns of Table VI do not give a true picture of the action of *F. graminearum*. The chief value of these columns is in demonstrating the increase in damage caused by *H. sativum* in naturally infested soil following wheat, *A. tenerum*, and *A. repens*. It can only be said that there is no definite evidence that the spread of *F. graminearum* in unsterilized soil is affected by the presence of susceptible plants in the soil.

TABLE V

Results of experiments on the spread of *O. graminis* in boxes of soil

Soil treatment	Distance from inoculum, in.	First replicate			Second replicate			Third replicate		
		Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations
Soil left bare. Soil + cornmeal, with no organism, placed at one end of box. Included to check against natural occurrence of *O. graminis* in soil.	0	26.9	0	–	26.6	0	–	23.7	0	–
	2	25.2	0	–	28.4	0	–	25.9	0	–
	4	22.1	0	–	27.1	0	–	21.9	0	–
	6	25.4	0	–	21.4	0	–	21.1	0	–
	8	25.9	0	–	23.4	0	–	21.1	0	–
	10	29.0	0	–	22.4	0	–	21.8	0	–
	12	23.5	0	–	20.6	0	–	21.6	0	–
	14	23.3	0	–	23.8	0	–	21.6	0	–
Soil left bare. *O. graminis* placed at one end of box.	0	26.1	14.5	+	20.4	15.0	+	25.9	13.3	+
	2	24.4	0	–	24.0	4.0	+	23.6	0	–
	4	25.6	0	–	24.1	0	–	21.9	0	–
	6	25.3	0	–	22.4	0	–	23.2	0	–
	8	26.0	0	–	22.8	0	–	27.4	0	–
	10	20.4	0	–	24.1	0	–	24.5	0	–
	12	22.5	0	–	21.2	0	–	22.6	0	–
	14	25.8	0	–	22.5	0	–	22.6	0	–
Soil seeded to wheat. *O. graminis* placed at one end of box.	0	29.2	26.7	+	26.7	10.0	+	30.9	4.0	+
	2	25.4	32.0	+	22.5	42.5	+	23.9	16.0	+
	4	18.2	38.2	+	21.4	24.0	+	20.5	26.2	+
	6	20.7	16.9	+	20.1	10.9	+	17.0	18.2	+
	8	22.0	11.4	–	21.6	8.3	+	22.4	3.1	+
	10	22.3	1.7	–	20.6	0	–	20.5	0	–
	12	20.7	0	–	19.9	0	–	22.9	0	–
	14	22.9	0	–	22.8	0	–	18.9	0	–
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of box.	0	27.2	5.4	+	24.9	14.7	+	25.0	7.3	+
	2	22.6	32.3	+	19.5	27.7	+	22.9	13.3	+
	4	22.2	10.0	+	20.6	13.3	+	20.9	9.1	+
	6	20.0	0	–	17.0	25.5	+	19.4	1.8	+
	8	19.3	0	–	17.4	17.8	+	21.2	2.8	+
	10	16.9	0	–	17.1	1.8	+	21.0	0	–
	12	18.4	0	–	20.6	1.5	+	17.1	0	–
	14	19.4	0	–	18.9	0	–	18.2	0	–
Soil seeded to *A. repens*. *O. graminis* placed at one end of box.	0	24.8	15.3	+	27.7	6.2	+	26.4	4.4	+
	2	29.6	26.7	+	26.5	22.5	+	23.5	2.2	+
	4	21.3	18.7	+	20.8	11.4	+	21.5	6.7	+
	6	21.6	0	–	20.1	3.3	–	20.5	0	–
	8	19.7	0	–	20.4	0	–	21.1	0	–
	10	21.7	0	–	20.6	0	–	20.5	0	–
	12	20.8	0	–	19.4	0	–	21.5	0	–
	14	16.5	0	–	18.5	0	–	19.1	0	–

Discussion

There is ample evidence that susceptible species tend to aid *O. graminis* to survive in the soil and to bring about an increase of the amount of inoculum of this organism for succeeding wheat crops. In the case of *H. sativum* the effects are somewhat less marked, but there is sufficient evidence to conclude that *Bromus inermis* as well as some species of *Agropyron* increase the amount

TABLE VI

RESULTS OF EXPERIMENTS ON THE SPREAD OF *F. graminearum* IN BOXES OF SOIL

Soil treatment	Distance from inoculum, in.	First replicate			Second replicate			Third replicate		
		Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations
Soil left bare. Soil + cornmeal, with no organism placed at one end of the box. Included to check against natural occurrence of *F. graminearum* in the soil.	0	27.4	0	− −	33.0	0	− −	30.8	2.7	− −
	2	23.9	0	− −	28.4	8.0	− H	28.0	5.5	− H
	4	22.9	0	− −	30.7	0	− −	30.5	3.6	− −
	6	18.5	0	− −	27.7	0	− −	29.9	2.9	− −
	8	23.0	0	− −	23.3	8.3	− H	27.5	5.7	− H
	10	24.6	0	− −	24.5	6.0	− H	25.0	8.9	− −
	12	22.7	1.4	− −	29.1	1.7	− −	29.3	4.4	− H
	14	24.4	0	− −	29.4	8.0	− H	26.9	2.2	− −
	16	23.6	0	− −	29.1	1.5	− −	30.1	2.5	− −
	18	24.4	0	− −	29.3	0	− −	27.0	10.0	− H
Soil left bare. *F. graminearum* placed at one end of the box.	0	24.6	5.0	+ −	26.3	20.0	+ H	34.1	13.3	+ −
	2	24.1	0	− −	29.9	9.3	− H	33.1	1.8	− −
	4	25.6	2.4	− H	27.3	0	− −	31.8	1.5	− −
	6	27.0	1.3	− −	26.7	5.7	− −	29.2	0	− −
	8	26.3	1.2	+ −	28.3	3.1	− H	27.1	0	− −
	10	26.0	8.3	− −	27.5	4.0	− H	29.0	2.2	− H
	12	24.2	0	− −	28.6	3.3	− −	27.0	0	− −
	14	25.8	3.6	+ −	27.3	8.6	− H	28.3	6.2	− H
	16	22.1	4.7	+ −	28.2	5.5	− H	27.4	0	− −
	18	24.8	0	− −	28.6	3.3	− H	27.9	0	− −
Soil seeded to wheat. *F. graminearum* placed at one end of the box.	0	23.5	29.1	+ −	27.9	37.5	+ H	32.7	10.0	+ H
	2	22.3	8.3	− H	30.3	29.3	− H	29.4	10.0	+ H
	4	21.4	14.0	− H	25.8	40.0	− H	27.9	8.9	− H
	6	19.3	9.1	− H	22.9	28.0	− H	26.1	12.3	− H
	8	21.3	10.0	− H	21.4	30.7	− H	23.8	14.3	− H
	10	20.7	8.6	− H	19.4	50.0	− H	22.5	9.2	− H
	12	18.8	7.5	− H	18.8	43.6	− H	19.4	12.0	− H
	14	17.6	11.7	− H	23.0	16.5	+ H	25.5	7.1	− H
	16	21.3	3.3	− −	20.6	35.7	− H	24.3	9.3	− H
	18	20.3	9.2	− −	20.2	36.4	− H	23.4	6.7	− H
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of the box.	0	22.3	16.0	+ H	24.5	49.1	+ H	32.3	18.3	− H
	2	24.4	20.0	+ H	24.9	35.6	+ H	29.5	12.3	− H
	4	23.7	18.7	+ H	24.4	40.0	+ H	28.4	12.9	− H
	6	20.2	25.0	− H	23.8	38.3	− H	26.3	15.0	− H
	8	23.2	10.0	− H	22.6	23.1	− H	22.0	17.3	− H
	10	22.2	0	− −	22.7	18.3	+ H	25.7	14.4	− H
	12	23.7	5.7	− H	24.2	11.4	+ H	24.5	16.0	− H
	14	22.8	7.3	− H	24.0	14.5	− H	25.1	10.9	− H
	16	21.9	1.8	− −	23.8	28.3	− H	21.7	20.0	− H
	18	21.7	12.0	− H	23.6	27.7	− H	23.0	12.0	− H
Soil seeded to *A. repens*. *F. graminearum* placed at one end of the box.	0	21.8	38.0	+ H	31.3	13.3	+ H	27.2	28.0	+ H
	2	22.5	15.6	+ H	25.0	36.9	− H	34.4	26.0	− H
	4	21.6	15.0	− H	27.7	21.4	− H	27.7	35.6	− H
	6	23.3	14.5	− H	25.1	48.3	− H	26.0	28.6	+ H
	8	17.0	40.0	− −	24.6	38.3	− H	25.3	22.9	− H
	10	22.5	21.4	− H	24.3	41.5	− H	27.7	15.0	− H
	12	19.9	32.0	− H	24.9	43.1	− H	27.0	18.0	− H
	14	22.9	11.1	− −	22.5	36.0	− −	25.1	18.3	− H
	16	21.6	4.0	− H	24.8	36.7	− H	25.9	13.3	− H
	18	21.8	6.2	+ H	20.8	31.1	− H	25.4	18.5	− H

of inoculum of this organism in the soil, and since the fungus seems to be of widespread distribution, it seems probable that such grasses will play a part in the foot rotting of wheat by this organism also. There is a fair amount of evidence that *Agropyron* species do not appreciably increase the amount of inoculum of *F. graminearum*. *Bromus inermis*, on the other hand, may be of importance. No evidence has yet indicated that resistant or immune species can be expected actually to have a detrimental effect on the foot-rotting fungi.

Survey work conducted in the province of Alberta has indicated that the damage caused by take-all is probably greater than that caused by other foot-rotting organisms, and for this reason special emphasis should be laid on the take-all problem. It is evident from the results that in order to control take-all, susceptible species of grasses should be avoided or used sparingly in rotations. Experimental results and field observations show that old stands of slender wheat grass have a marked effect upon the accumulation of *O. graminis*, and for this reason cultivation of this crop should not be encouraged where the take-all disease presents a very serious problem in wheat culture. It has been seen that *A. repens* occurs often even in fallow fields where other weeds have been kept under control. Rotations which do not involve eradication of this grass, and summerfallow methods which do not destroy it, cannot be expected to solve the problem, and may even intensify it. From knowledge so far accumulated it would seem that awnless brome grass may perhaps offend less in this respect than slender wheat grass, but it certainly is not entirely free from blame.

The experiments shed light on the manner in which *O. graminis* spreads in the soil. This organism spreads little, if at all, in bare soil, but in soil supporting growth of susceptible grasses it spread a considerable distance. It is true that the greatest spread which occurred was only twelve inches in six weeks, but when it is considered that during this short period it had to establish itself upon healthy young plants in the soil and pass from plant to plant presumably by means of the fine network of roots, that no attempt was made to provide ideal conditions for the growth of the organism in the soil, that previous attempts to demonstrate the spread of the organism had resulted in failure, and that in the unplanted soil the degree of infection of wheat seedlings grown actually in the inoculum six weeks after it was applied was much lighter than that of plants two and four inches distant in planted soil, there is abundant evidence that susceptible species aid greatly in the spread of the organism, if, indeed, they are not essential.

It is of interest to compare this lateral growth of *O. graminis* with that of *Phymatotrichum omnivorum* (15), which has been found to spread radially in all directions from infected cotton plants at the astounding rate of 4½ metres in 50 days. Recently it has been demonstrated at the Rubber Research Institute of Malaya (19) that rhizomes of the rubber root-rot fungi *Fomes lignosus*, *Ganoderma pseudoferreum* and *Fomes noxius* do not grow directly through the soil, but require as a vehicle a chain of solid surfaces, preferably the surfaces of roots. There appears to be much in common among these various parasitic fungi which have such widely different hosts.

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References

1. BRITTLEBANK, C. C. Green manurial crops and "take-all". Victoria (Australia) Agr. Dept. J. 17 : 171-174. 1920.
2. BROADFOOT, W. C. Studies on foot and root rot of wheat. II. Cultural relationships on solid media of certain micro-organisms in association with *Ophiobolus graminis* Sacc. Can. J. Research, 8 : 545-552. 1933.
3. BROADFOOT, W. C. Studies on foot and root rot of wheat. III. Effect of crop rotation and cultural practice on the development of foot rot of wheat. Can. J. Research, 10 : 95-114. 1934.
4. CHRISTENSEN, J. J. Studies on the parasitism of *Helminthosporium sativum*. Univ. Minn. Agr. Expt. Sta., Tech. Bull. 11. 1922.
5. DAVIS, R. J. Studies on *Ophiobolus graminis* Sacc. and the take-all disease of wheat. J. Agr. Research, 31 : 801-825. 1925.
6. DRESCHLER, C. Some graminicolous species of *Helminthosporium*. J. Agr. Research, 24 : 641-740. 1923.
7. FELLOWS, H. Studies of certain soil phases of the wheat take-all problem. (Abstract) Phytopathology, 19 : 103. 1929.
8. FRAZIER, W. C. and FRED, E. B. Movement of legume bacteria in soil. Soil Sci. 14 : 29-36. 1922.
9. GARRETT, S. D. Factors affecting the severity of take-all. J. Dept. Agr. South Australia, 37 : 664-674. 1934.
10. GREANEY, F. J. and BAILEY, D. L. Root-rots and foot-rots of wheat in Manitoba. Dept. Agr. Dom. of Canada, Bull. 85, n.s. 1927.
11. HENRY, A. W. Root-rots of wheat. Univ. Minn. Agr. Expt. Sta. Tech. Bull. 22. 1924.
12. HENRY, A. W. The natural microflora of the soil in relation to the foot-rot problem of wheat. Can. J. Research, 4 : 69-77. 1931.
13. HENRY, A. W. Occurrence and sporulation of *Helminthosporium sativum* P.K.B. in the soil. Can. J. Research, 5 : 407-413. 1931.
14. HENRY, A. W. Influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis* Sacc. Can. J. Research, 7 : 198-203. 1932.
15. KING, C. J. Habits of the cotton rootrot fungus. J. Agr. Research, 26 : 405-418. 1923.
16. KIRBY, R. S. The take-all disease of cereals and grasses. Phytopathology, 12 : 66-88. 1922.
17. KIRBY, R. S. The take-all disease of cereals and grasses caused by *Ophiobolus cariceti*. Cornell Agr. Expt. Sta. Mem. 18. 1925.
18. MCNAMARA, H. C. and HOOTON, D. R. Studies of cotton root rot at Greenville, Texas. U.S. Dept. Agr. Circ. 85. 1929.
19. NAPPER, R. P. N. Root disease investigations. Rubber Research Inst. Malaya, Ann. Rept. for 1933 : 105-111. 1934.
20. PADWICK, G. W. and HENRY, A. W. The relation of species of *Agropyron* and certain other grasses to the foot-rot problem of wheat in Alberta. Can. J. Research, 8 : 349-363. 1933.
21. ROSEN, H. R. and ELLIOTT, J. A. Pathogenicity of *Ophiobolus cariceti* in its relationship to weakened plants. J. Agr. Research, 25 : 351-358. 1923.
22. RUSSELL, R. C. Take-all studies. Rept. Dom. Botanist (Canada) for 1927 : 105-109. 1928.
23. RUSSELL, R. C. Take-all studies. Rept. Dom. Botanist (Canada) for 1928 : 96-107. 1929.
24. RUSSELL, R. C. Field studies of take-all in Saskatchewan. Sci. Agr. 10 : 654-668. 1930.
25. RUSSELL, R. C. Studies in cereal diseases. X. Studies of take-all and its causal organism, *Ophiobolus graminis* Sacc. Dom. Canada, Dept. Agr. Bull. 170 n.s. 1934.

26. SANFORD, G. B. Report of the Dominion Field Laboratory of Plant Pathology, Edmonton, Alberta. Foot rots of spring wheat. Rept. Dom. Botanist (Canada) for 1928 : 108-112. 1929.

27. SANFORD, G. B. and BROADFOOT, W. C. Studies of the effects of other soil-inhabiting micro-organisms on the virulence of *Ophiobolus graminis* Sacc. Sci. Agr. 11 : 512-528. 1931.

28. SEWELL, M. C. and MELCHERS, L. E. The effect of rotation and tillage on foot-rot of wheat in Kansas, 1920-1924. J. Am. Soc. Agron. 16 : 768-771. 1924.

29. STAKMAN, L. J. A *Helminthosporium* disease of wheat and rye. Univ. Minn. Agr. Expt. Sta. Bull. 191. 1920.

30. TAUBENHAUS, J. J., DANA, B. F. and WOLFF, S. E. Plants susceptible or resistant to cotton root rot and their relation to control. Texas Agr. Expt. Sta. Bull. 393. 1929.

31. TAUBENHAUS, J. J. and KILLOUGH, D. T. Texas root rot of cotton and methods of its control. Texas Agr. Expt. Sta. Bull. 307. 1923.

32. WATERS, R. Take-all disease in wheat. Incidence in New Zealand. New Zealand J. Agr. 20 : 137-143. 1920.

REACTION OF BARLEY VARIETIES TO INFECTION WITH COVERED SMUT (*USTILAGO HORDEI* PERS. K & S)[1]

By O. S. Aamodt[2] and W. H. Johnston[3]

Abstract

Results are presented of tests conducted at the University of Alberta, Edmonton, during the years 1931-34, to determine the relative resistance of barley varieties to the covered smut disease caused by *Ustilago hordei*.

Extensive field trials, including 138 varieties, carried out in 1931, with hulled seed, gave inconclusive results owing to low infection percentages. Junior and Eureka, two naturally hulless varieties, evidenced high susceptibility with 66 and 42% infection respectively. Field tests of a number of standard varieties in 1932, in which the seed was dehulled with sulphuric acid, resulted in an increase in the percentage of smutted plants. Unfortunately, the acid treatment of the kernels caused a general impairment in germination which lessened somewhat the significance of the results obtained. In 1934, the comparative infections and stands of varieties grown from hulled, hand-dehulled, scarified and acid-dehulled seed were determined. The data were treated statistically by the analysis of variance method. Significant variations due to varieties, treatments and interaction of varieties and treatments were obtained with regard to both percentage infection and percentage stand. All three of the dehulling measures increased smut infection significantly. Highest infection percentages resulted from hand-dehulled seed, followed by acid-dehulled and scarified seed in the order mentioned.

The least reduction in stand resulted from hulled seed and the greatest from acid-dehulled seed. Scarified and hand-dehulled seed gave stands intermediate in numbers. Distinct varietal differences existed in thickness of hull or in the resistance of the hull to acid treatment. The average percentage stands of the different varieties tended to be directly proportional, and the average percentage smut infection inversely proportional to the amount of hull remaining on the kernels following acid treatment. Varieties grown from acid-dehulled and scarified seed were found to be delayed in heading 1½ and 2½ days respectively as compared with varieties grown from hulled or hand-dehulled seed. There was a tendency for the later varieties to be more susceptible to covered smut than the earlier ones.

The varieties used in these investigations differed greatly in their reaction to covered smut. A fair degree of correlation was found to exist between the varietal infection percentages induced in 1932 by acid-dehulled seed and those induced by either hand-dehulled or acid-dehulled seed in 1934.

Two distinct physiologic forms of *U. hordei* were found in collections gathered from six points in central Alberta. These are readily distinguished by their reaction on the varieties Eureka and Canadian Thorpe or Hannchen.

From the experimental data it was concluded that the following varieties showed resistance to covered smut:

Six-rowed, hulled types—O.A.C. No. 21, Atlas, Sacramento, Glabron, Velvet, Leiorrhynchum, Wisconsin Barbless No. 38, Shaw, Sol and Success.

Two-rowed, hulled types—Spartan, Golden Pheasant and Horn.

Hulless types—Himalayan, New Era, Russian, Mongolian and Burbank.

The following varieties showed susceptibility to one or more of the smut collections used:

Six-rowed, hulled types—Bearer, Lapland, Star, Manchurian, Peatland, Trebi, Silver King, Vaughn, Comfort, Regal, Newal and Colsess.

Two-rowed, hulled types—Binder, Canadian Thorpe, Duckbill, Gold, Hannchen, Swanneck and Charlottetown.

Hulless types—Junior, Eureka, Improved White Hulless and Trifurcatum.

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Introduction

For some time the Department of Field Crops, University of Alberta, has been testing the reaction of barley varieties to infection with the more common diseases of the crop occurring in western Canada, in order that improvement of already existing strains may be undertaken. This paper summarizes the results obtained in regard to varietal reaction to infection with covered smut, *Ustilago hordei*.

The losses from the barley smuts in Canada are much greater than is generally appreciated. During the period 1920-23 Canadian farmers lost over one million dollars annually from these diseases (8). Covered smut (*U. hordei*) is a widespread disease of the barley crop in western Canada, but appears to be particularly destructive in Alberta (3, 4, 13). In 1931, 53% of the fields examined in Alberta were smutty; the average damage being 2.3%. The two highest infections reported were 25 and 70% (3). Covered smut was present in 33% of the fields examined in 1932 and 1933. The average damage in infested fields was 2.9% in 1932 and 1.9% in 1933. Infections of 15 and 30% were recorded in 1932, while the highest infection reported for 1933 was 10% (3).

Varietal Reaction

Literature Review

The literature concerning varietal resistance to the covered smut disease is comparatively fragmentary, and the results reported largely inconclusive. Briggs (1) refers to extensive tests carried out at the California Agricultural Experiment Station in which practically no infection resulted, although the seed had been heavily inoculated with smut. Tisdale (15) in Virginia was unable to determine the resistance of varieties to covered smut because of a lack of satisfactory infection. Conners (2) also reports inconclusive results after testing over a hundred varieties and strains. Rodenhiser (14) refers to investigations carried out at University Farm, St. Paul, Minnesota, during the years 1924 to 1927, in which about 135 varieties and selections of barley were treated for their reaction to covered smut. Although the varieties were heavily inoculated, so little smut developed in most of them that the results could not be considered significant. Observations made at about the same time, on the reaction of varieties in other plots at the Minnesota Agricultural Experiment Station, and at a number of its Branch Stations, yielded more definite information regarding varietal resistance to the covered smut disease. Lion proved immune, and White Spring Hulless was highly susceptible. The varieties Odessa, Trebi, Glabron and Svanhals showed moderate susceptibility, while Manchuria and Svansota were classed as resistant. Hanna and Popp (9), working at Winnipeg, Manitoba, have contributed some definite information regarding the reaction to *U. hordei* of four varieties commonly grown in western Canada. They report that the varieties Hannchen, Canadian Thorpe and Trebi evidenced susceptibility to the collection of smut used, while O.A.C. No. 21 was free from smutted heads.

As far as could be determined from the literature, the above-mentioned investigators used inoculum with a high degree of viability. It is therefore evident that other factors, about which little seems to be known, are important contributing factors influencing successful infection of barley. Tisdale (17) showed that greatly increased infection may be secured by removing the hull prior to inoculation. This finding was corroborated by Faris (6) and Briggs (1). Faris (5) demonstrated that high infection could be obtained on the Hannchen variety at soil moistures and temperatures generally existing at the time of seeding. He states, furthermore, that the biologic form of the smut used may have more bearing on successful infection than any soil conditions. Taylor and Zehner (16) found that in winter barley greater covered smut infection resulted from deep than from shallow seeding. Rump, cited by Leukel (12) found that an alkali soil stimulated covered smut development, that acid soil was injurious to it and that a soil moisture content of 20% was the optimum for development of covered smut. Schaffnit, also cited by Leukel (12), reported that a soil rich in organic matter and carbonic acid favored covered smut infection.

VARIETAL TESTS, 1931

Methods

Extensive tests to determine the resistance of barley varieties to covered smut were made in 1931. No attempt was made to remove the hull in the case of hulled varieties.* Approximately 75 inoculated seeds of each variety were sown in 10-foot rows. The inoculum consisted of a composite sample of chlamydospores gathered in 1930 from the rod-row varietal plots. The smutted heads were run through an ordinary meat grinder, and the powdered spores dusted on the seed just previous to sowing. All seeding was done in the latter half of May after the soil had reached a temperature of approximately 15-20° C. The first test included 138 varieties and strains from the barley classification nursery grown at the University of Alberta.

Experimental Results

The results of this experiment are in accord with those reported by earlier workers in that, owing to low infection, it is difficult to draw definite conclusions regarding varietal reaction. The infection percentages for 32 of the 138 varieties in this test are given in Table I as "Experiment A". The only variety showing extreme susceptibility was Junior, with 66% infection. Other varieties exhibiting more than 10% infection are listed below, with their respective infection percentages:

Six-rowed hulless types—Trifurcatum, 21%; Eureka, 18%; Improved White Hulless, 14%.

Six-rowed hulled types—Silver King, 15%; Garton's No. 68, 11%; Sans Barb Early, 11%.

Two-rowed hulled types—Alpha, 13%; Danish Island, 12%.

* *Throughout this paper, the current practice is being followed of referring to "hulled" seed or varieties as normal grain with the hull intact; "hulless" as normal grain without the hull attached to the caryopsis, and "dehulled" as seed of hulled varieties from which the hulls have been removed artificially.*

Trebi and O.A.C. No. 21, two commonly grown six-rowed barleys, showed only 2% infection. The two-rowed barleys, Canadian Thorpe and Hannchen, exhibited 5 and 9% covered smut respectively. Of the smooth-awned varieties tested, Glabron, Velvet and Regal failed to show any smutted plants, while Comfort had 5%.

TABLE I

REACTION OF SOME COMMON BARLEY VARIETIES TO INFECTION WITH COVERED SMUT (*U. hordei*) AS DETERMINED BY FIELD TESTS CONDUCTED IN 1931 AT THE UNIVERSITY OF ALBERTA, EDMONTON

Variety and group	Number*	Percentage of plants infected	
		Experiment A	Experiment B
Six-rowed, hulled, rough-awned types			
Atlas	C.A.N. 702	–	0
Bark's	C.A.N. 703	0	1
California Mariout	C.A.N. 1083	0	0
Garton's No. 68	C.A.N. 1033	11	–
Manchurian	C.A.N. 726	5	7
Minsturdi	C.A.N. 732	2	0
O.A.C. No. 21	C.A.N. 734	2	0
Peatland	C.A.N. 722	2	15
Sacramento	C.A.N. 744	–	0
Silver King	C.A.N. 1048	15	–
Star	C.A.N. 748	0	5
Trebi	C.A.N. 753	2	4
Vaughn	C.A.N. 759	–	0
Vaughn	C.A.N. 1090	0	0
Six-rowed, hulled, smooth-awned types			
Comfort	C.A.N. 712	5	0
Glabron	C.A.N. 718	0	0
Regal	C.A.N. 742	0	0
Sans Barb Early	N.S.N. I-31-7	11	–
Newal	C.A.N. 1089	–	2
Velvet	C.A.N. 755	0	0
Six-rowed, hulled, hooded types			
Colsess	C.A.N. 772	4	2
Sol	C.A.N. 782	–	6
Six-rowed, hulless types			
Eureka	C.A.N. 773	18	42
Hulless	N.S.N. I-31-6	–	0
Improved White Hulless	C.A.N. 1056	14	–
Junior	C.A.N. 786	66	–
New Era	C.A.N. 721	–	3
Trifurcatum	C.A.N. 891	21	–
Two-rowed types			
Alpha	C.A.N. 801	13	–
Alberta Beardless (hooded)	C.A.N. 874	8	4
Canadian Thorpe	C.A.N. 816	5	6
Charlottetown No. 80	C.A.N. 817	4	3
Danish Island	C.A.N. 1002	12	–
Duckbill	C.A.N. 826	2	12
Gold	C.A.N. 829	2	6
Hannchen	C.A.N. 837	9	7
Horn	C.A.N. 1078	0	0
Plumage Archer	C.A.N. 1004	0	4
Spartan (smooth awn)	C.A.N. 860	0	3

**C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

A second experiment, "B", was conducted in which the reaction to covered smut infection was determined for 31 of the standard varieties. These varieties, together with their infection percentages, are given in Table I as "Experiment B".

Eureka, a hooded, hulless variety, showed considerable susceptibility with 42% infection. Peatland, a six-rowed, hulled, bearded type, and Duckbill, a two-rowed, hulled, bearded type, were the only other varieties displaying susceptibility. No smutted plants were found in O.A.C. No. 21, Atlas, Minsturdi, Sacramento, Vaughn, Glabron, Velvet and Regal. Canadian Thorpe and Hannchen showed 6 and 7% infection respectively.

The relatively high infection resulting on the naturally hulless varieties indicated that both inoculum and environmental conditions were favorable for successful infection. Furthermore, what is more important, it suggests that the presence of the hull was possibly a deterrent to successful infection in the case of hulled varieties. To investigate this possibility, a second varietal test was conducted in 1932, in which the seed was dehulled.

Varietal Tests, 1932

Methods

The kernels of the varieties used in this test were dehulled by means of sulphuric acid. The feasibility of using this chemical to dehull barley kernels in order to induce infection with covered smut, was suggested by Briggs (1). The technique used in dehulling the kernels in these experiments was one developed by the junior author while studying the inheritance of reaction to covered smut infection in certain barley crosses (11).

The kernel lots were immersed in concentrated sulphuric acid for a period of two minutes. Following this they were rinsed with cold water and returned to the acid for 10 seconds. The action of the acid on the wet seed coats caused a rapid and more or less complete removal of the hull from the greater part of the kernel. The dehulled kernels were then washed thoroughly with cold water for two or three minutes. In an effort to remove all traces of acid, the kernel lots were placed in a concentrated solution of $NaHCO_3$ (baking soda) for several minutes. After another rinse with cold water they were placed on blotting paper to dry.

Two series of approximately 50 kernels of each variety were dehulled by acid. One series was inoculated with a composite of covered smut chlamydospores collected from the varietal plots at Edmonton in 1931. The other was dusted with chlamydospores from a collection made in a commercial field of smooth-awned barley at Winterburn, Alberta.

The inoculated seed of both replicates was sown in 10-foot rows, with soil temperatures of approximately 16° C. Unfortunately, the viability of the acid-treated seed was generally low; this being reflected in reduced stands. The kernels of some of the two-rowed barleys appeared especially susceptible to acid injury. For this reason it was necessary to include in the data the total number of plants, in addition to the percentage infected.

Experimental Results

The data in Table II summarize the reaction of 32 varieties exposed to both the Edmonton and Winterburn smut collections. To facilitate examination and discussion, the varieties have been grouped into several commonly

TABLE II

REACTION OF SOME COMMON BARLEY VARIETIES TO TWO COLLECTIONS OF COVERED SMUT, WHEN GROWN FROM SEED DEHULLED WITH SULPHURIC ACID, AND SOWN IN THE FIELD AT THE UNIVERSITY OF ALBERTA, EDMONTON, 1932

Variety and group	Number*	Smut from			
		Edmonton		Winterburn	
		Total number of plants	Percentage of plants infected	Total number of plants	Percentage of plants infected
Six-rowed, hulled, rough-awned types					
Bearer	C.A.N. 704	23	9	10	0
Lapland	N.S.N. I-32-2	32	19	29	31
Manchurian	C.A.N. 726	29	55	14	14
Minsturdi	C.A.N. 732	26	8	18	0
O.A.C. No. 21	C.A.N. 734	42	2	27	11
Peatland	C.A.N. 722	17	24	25	16
Sacramento	C.A.N. 744	10	0	14	0
Star	C.A.N. 748	10	20	12	25
Trebi	C.A.N. 753	36	25	27	11
Vaughn	C.A.N. 759	29	21	21	5
Six-rowed, hulled, smooth-awned types					
Comfort	C.A.N. 712	41	10	35	0
Glabron	C.A.N. 718	38	0	42	0
Newal	C.A.N. 1089	25	28	24	25
Regal	C.A.N. 742	42	17	27	7
Velvet	C.A.N. 755	36	0	28	0
Wisconsin Barbless No. 38	C.A.N. 758	23	9	22	0
Six-rowed, hulled, hooded types					
Colsess	C.A.N. 772	35	9	25	4
Sol	C.A.N. 782	16	13	10	0
Success	N.S.N. I-32-4	26	0	13	0
Six-rowed, hulless types					
Eureka	C.A.N. 773	65	15	50	0
Himalayan	N.S.N. I-32-3	54	2	32	0
New Era	C.A.N. 721	58	5	50	0
Two-rowed types					
Alberta Beardless (hooded)	C.A.N. 874	12	8	14	0
Binder	N.S.N. I-32-8	32	0	25	0
Canadian Thorpe	C.A.N. 816	14	7	13	23
Charlottetown No. 80	C.A.N. 817	20	45	24	29
Duckbill	C.A.N. 826	10	30	12	33
Gold	C.A.N. 829	9	11	17	41
Golden Pheasant	N.S.N. I-32-9	28	0	19	0
Hannchen	C.A.N. 837	14	36	7	0
Horn	C.A.N. 1078	48	2	21	10
Spartan (smooth-awned)	C.A.N. 860	15	13	14	29

* *C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

recognized classes. In general, the infection percentages of varieties with stands of less than 20 plants have been considered as too unreliable to merit consideration. As has been indicated previously, the individual plant was used as the basis for the calculation of infection percentages.

A general examination of the data in Table II shows that the infection resulting from the Edmonton collection of smut is generally more severe than that resulting from the Winterburn collection. The average percentage infection of the 32 variteies when inoculated with the Edmonton smut collection was 13.8; with the Winterburn collection it was 9.5.

Reaction of six-rowed, hulled, rough-awned types. Manchurian, with 55% infection, was the most susceptible to the Edmonton sample of smut. Trebi, Vaughn and Lapland displayed moderate susceptibility to the smut, with infection percentages ranging from 19 to 25. Lapland showed considerable susceptibility to the Winterburn collection, while Peatland, O.A.C. No. 21 and Trebi were moderately so. Bearer appeared highly resistant to both collections. No smutted plants were found in either replicate of Sacramento, but the reduced population in these instances makes any definite statement regarding resistance unreliable.

Reaction of six-rowed, hulled, smooth-awned types. High resistance to both collections of smut was displayed by Glabron and Velvet, while Newal and Regal proved susceptible. Comfort and Wisconsin Barbless No. 38 showed moderate resistance to the Edmonton collection of smut, and produced no smutted plants when inoculated with the Winterburn collection.

Reaction of six-rowed, hulled, hooded types. Colsess and Sol were moderately resistant, and Success was completely resistant to the covered-smut collections used.

Reaction of six-rowed, hulless types. The varieties New Era and Himalayan displayed high resistance to both collections of smut used. Eureka proved to be moderately susceptible to the Edmonton collection and highly resistant to the one from Winterburn.

Reaction of two-rowed types. No smut appeared on either replicate of Binder and Golden Pheasant. Horn appeared to be highly resistant to the Edmonton collection but only moderately so to the one from Winterburn. Charlottetown, on the other hand, showed high susceptibility to both, with infections of 45 and 29%. The varieties Hannchen, Duckbill, Gold, Canadian Thorpe and Spartan appeared to be susceptible to one or both of the collections used; but again inadequate populations render a definite statement regarding resistance impossible.

No smut tests were made on hulled seed in 1932, consequently it is difficult to state how much the dehulling increased the susceptibility of the various varieties to infection. The results do indicate, however, that considerably higher smut percentages are obtained when the hull is partially removed by acid. This method of dehulling resulted in reduced stands, which lessened

somewhat the significance of the data obtained. In general the results of these experiments are in accord with those of earlier workers (1, 15), in that results obtained from extensive barley smut tests, using hulled seed, are not entirely satisfactory.

In order to obtain further information on the best method to use in dehulling seed, and its effectiveness in increasing smut infections of various barley varieties, another experiment was conducted in 1934.

Varietal Tests, 1934

Methods

In 1934, the reaction to covered smut was determined for 25 hulled varieties of barley. Three separate methods of dehulling the kernels were employed:

i. Hand removal of hull over the region of the embryo with a scalpel.

ii. Scarification with sandpaper. This was accomplished by rubbing the seed between two sheets of sandpaper of medium coarseness. This method of dehulling removed the hull quite effectively from the dorsal and ventral sides of the kernels, but did not expose the embryos.

iii. Digestion of hull with concentrated sulphuric acid. The details of the method employed in this instance were similar to those used in dehulling the kernels in the 1932 tests.

Hulled samples of each variety were also prepared to serve as checks, making in all four series of kernels.

Duplicate lots of 75 kernels were used in each treatment and the plots were systematically arranged. The inoculum consisted of a composite sample of smutted spikes obtained from the 1933 varietal plots. Again the plant, rather than the spike, was used as the basis of determining infection percentages. All plants showing any degree of infection were considered susceptible.

Experimental Results

Before considering the specific varietal reaction to covered smut indicated in this trial, it is proposed to discuss the relative merits of the four methods of treating the seed prior to inoculation as outlined in the previous section. Special attention will be given their relative values in inducing smut infection, their effect on emergence or final stand, and their effect on plant development. A considerable number of correlation coefficients were calculated in order to show the similarity of the different treatments on the varieties, and to indicate the reliability of the results. For the sake of convenience and clarity each pair of variables correlated has been assigned a number and is listed in numerical order in Table VI. Reference will be made by number to any correlation under discussion.

Effect of dehulling on covered smut infection. The average infection percentages of the different varieties for each of the four treatments is given in Table III. It is evident that much lower infections resulted from hulled

TABLE III

AVERAGE PERCENTAGE SMUT INFECTION WITH *U. hordei* AND AVERAGE PERCENTAGE STANDS OF 25 VARIETIES GROWN FROM HULLED, HAND-DEHULLED, SCARIFIED AND ACID-DEHULLED SEED IN 1934

Variety	Number*	Average percentage stand and infection with *U. hordei*								Mean percentage stand (8 plots)	Mean percentage infection (8 plots)
		Hulled		Hand-dehulled		Scarified		Acid-dehulled			
		Stand	Infection	Stand	Infection	Stand	Infection	Stand	Infection		
Six-rowed, hulled, rough-awned types											
Atlas	C.A.N. 702	87	0	80	1	85	0	75	1	81.6	0.5
Bearer	C.A.N. 704	95	2	75	26	68	3	49	28	74.5	14.4
Lapland	N.S.N. I-32-2	71	12	39	45	35	31	17	32	40.7	29.6
Manchurian	C.A.N. 726	93	8	51	53	40	28	15	46	49.4	33.5
O.A.C. No. 21	C.A.N. 734	89	1	80	1	56	3	41	4	66.3	2.1
Peatland	C.A.N. 722	84	1	76	22	48	18	23	16	57.4	14.1
Trebi	C.A.N. 753	83	1	76	35	77	16	63	21	74.3	18.1
Vaughn	C.A.N. 759	81	5	61	4	60	0	57	8	64.9	4.3
Six-rowed, hulled, smooth-awned types											
Comfort	C.A.N. 712	81	3	61	21	43	2	61	6	61.3	7.6
Glabron	C.A.N. 718	96	0	76	9	71	2	67	1	77.4	3.0
Newal	C.A.N. 1089	91	4	36	31	57	12	56	7	60.0	13.3
Leiorrhynchum	N.S.N. I-32-11	89	0	40	3	77	4	68	4	68.1	2.8
Regal	C.A.N. 742	81	0	69	9	63	9	57	8	67.4	6.4
Velvet	C.A.N. 755	91	1	71	6	55	0	55	3	67.6	2.3
Wisconsin Barbless No. 38	C.A.N. 758	77	0	69	1	65	0	60	0	67.9	0.3
Six-rowed, hulled, hooded types											
Colsess	C.A.N. 772	81	5	68	39	67	21	33	33	61.8	24.4
Shaw	C.A.N. 1047	84	1	51	11	53	9	27	14	53.5	8.5
Sol	C.A.N. 782	77	3	52	9	39	12	39	4	50.1	6.8
Two-rowed, hulled types											
Binder	N.S.N. I-32-8	91	1	76	46	72	15	47	20	71.0	20.1
Canadian Thorpe	C.A.N. 816	88	1	79	33	53	24	32	40	62.5	24.5
Duckbill	C.A.N. 826	92	11	77	21	68	24	57	4	73.4	14.6
Gold	C.A.N. 829	84	2	79	50	59	10	33	49	63.4	27.5
Hannchen	C.A.N. 837	87	6	87	60	80	19	41	43	73.1	31.8
Spartan	C.A.N. 860	85	0	68	9	51	3	43	5	61.3	4.3
Swanneck	N.S.N. I-33-1	95	1	55	13	61	7	41	21	62.8	10.3
Mean (50 plots)		85.7	2.6	65.8	22.2	59.8	10.7	46.1	16.5		

**C.A.N.* = *Canadian Accession Number; N.S.N.* = *University of Alberta Nursery Stock Number.*

seed than from seed receiving any of the dehulling treatments. Hand-dehulling proved superior to either scarification or dehulling by acid in inducing infection. The mean percentage of infection of the hulled series was 2.6; of the hand-dehulled, 22.2; of the scarified, 10.7; and of the acid-dehulled, 16.5.

The data were analyzed by the analysis of variance method (7, 10). For this purpose duplicate plots of each variety for each treatment were used, making in all 200. The summarized results are given in Table IV.

TABLE IV

THE ANALYSIS OF VARIANCE FOR SMUT INFECTION

Variations due to	D/F	Sum of squares	Mean square	Standard deviation	$\frac{1}{2}$ log e of mean square	Z
Varieties	24	21227.73	884.49	—	3.39	1.58*
Treatments	3	10582.02	3527.34	—	4.08	2.50*
Varieties × treatments	72	11542.23	160.31	—	2.54	0.73*
Replicates†	4	218.52	54.63	—	2.00	0.19**
Error	96	3605.48	37.56	6.13	1.81	
Total	199	47175.98				

**Value of Z exceeds the 1% point.*
***Variance not significant.*
†Degrees of freedom include 1 for replicates and 3 for interaction replicates × treatments.

It will be noted that no significant variation due to replicates was found. This indicates that, while the plots had been arranged in a systematic rather than a randomized order, the significance of the results is little affected. It would appear that soil heterogeneity is not an important factor influencing infection from seed-borne pathogens as exemplified by *U. hordei*. Hence randomization of the plots is not essential for a study of this character. Consequently the method of analysis of variance is applicable to the data being presented. The degree of association between infection percentages shown by varieties contained in the duplicate rows of each treatment is given by correlations 1, 2, 3 and 4 (Table VI). It is evident that comparatively high correlations exist between replicates of varieties grown from seed receiving any one of the three dehulling treatments, while one of considerably lower value exists in the case of hulled seed. The agreement was especially high in the hand-dehulled seed where $r = 0.853$. The corresponding values for the scarified and acid-dehulled series were $r = 0.706$ and $r = 0.675$ respectively. These high correlations between replicates of varieties grown from dehulled seed clearly indicate the high agreement between replicates, and reflect favorably on the significance of the data obtained. The correlation coefficient of 0.476, obtained between duplicate values of the hulled series, while mathematically significant in the light of its P value, is of little value in this study owing to the low infection percentages obtained. The magnitude of the correlation value in question was largely determined by

the infection percentage derived from only four of the 25 varieties tested. The same criticism probably applies to all correlation values involving the infection percentages obtained from hulled seed.

The mean square for varieties was highly significant, indicating that the average infections of the varieties tested were significantly different in all tests. A detailed discussion of varietal reaction will be given in a later section of this paper.

The mean square for treatment was also found to be significantly higher than that for error, showing that the average infection percentages induced by the different treatments differed significantly. The standard error of the difference between two 50-plot means would be $6.13\ \sqrt{2}\,/\,\sqrt{50}$ or 1.23% smut infection. Accepting twice the standard error of the difference as a convenient minimum level of significance, it follows that when the difference between two means exceeds 2×1.23, or 2.46% infection, the chances are greater than 19 : 1 that this difference was not due to chance. From Table III it may be seen that the mean infection resulting from each treatment was as follows: hulled—2.6%; hand-dehulled—22.2%; scarified—10.7%; acid-dehulled—16.5%. It is evident that all four treatments differ significantly in their capacity to induce smut infection.

It has been demonstrated that significant differences in degree of infection exist for both varieties and treatments. The question now arises whether or not these varieties responded similarly in all treatments. The significant mean square of interaction of varieties and treatments shows that some varieties acted in a differential manner in certain of the treatments. A rough comparison of the relative response of the different varieties to the different treatments may be obtained by correlating the average infection percentages of the varieties of one treatment with those of another (correlations 5, 6, 7, 8, 9 and 10, Table VI). The average varietal infections resulting from acid-dehulled seed gave a slightly better agreement with those occurring from hand-dehulled seed than did the corresponding values induced by scarification. In the first instance $r = 0.854$ and in the second, $r = 0.748$. The comparable value between hulled and hand-dehulled seed was considerably lower, r being 0.522.

Effect of dehulling on stand of plants. The average percentage stands of the varieties grown from hulled and dehulled seed are given in Table III. As far as could be observed, the final stand of any variety was a direct reflection of its emergence; all emerged seedlings reaching maturity. It is evident from the data in Table III that the highest stands resulted from hulled seed and the lowest from acid-dehulled seed. Scarification and hand-dehulling gave stands intermediate in number. The average percentage stand of all varieties from hulled seed was 86%; from hand-dehulled seed, 66%; from scarified seed, 60%; and from acid-dehulled seed, 46%. The analysis of variance method was applied also to the data concerning stand of plants. The results are given in Table V.

It will be noted that again no significant variation exists in the case of replicates, demonstrating that any different arrangement of the plots would have had little influence on the significance of the results obtained. The agreement between replicates of the different treatments in regard to percentage stand is shown by correlations 11, 12, 13 and 14 (Table VI). The best agreement existed in the cases of hand-dehulled and scarified lots where correlation coefficients of 0.705 and 0.692 respectively were obtained. In the two replicates from acid-dehulled seed $r = 0.559$, and in the hulled seed $r = 0.403$.

TABLE V

THE ANALYSIS OF VARIANCE FOR PERCENTAGE STAND OF PLANTS

Variation due to	D/F	Sum of squares	Mean square	Standard deviation	$\frac{1}{2}$ log e of mean square	Z
Varieties	24	20514.22	854.76	—	3.38	1.06*
Treatments	3	40679.30	13559.73	—	4.76	2.44*
Varieties × treatments	72	12562.70	174.48	—	2.58	0.26*
Replicates**	4	227.40	56.85	—	—	
Error	96	9910.60	103.24	10.16	2.32	
Total	199	83894.22				

**Value of Z exceeds the 1% point.*

***Degrees of freedom include 1 for replicates and 3 for interaction of replicates × treatments.*

The mean square due to varieties is significantly larger than that due to error, indicating that on the average significant differences exist in the capacity of varieties to emerge following the treatments given. The standard error of the difference between two 8-plot means is $10.16\sqrt{2}/\sqrt{8}$ or 5.08%. Differences in mean stand of 10.16% may be judged significant. From the mean percentage stands of the different varieties for all treatments given in Table III it may be seen, for example, that Atlas shows a significantly higher percentage stand than O.A.C. No. 21, and O.A.C. No. 21 in turn, a significantly higher percentage stand than either Lapland or Manchurian. Similarly, the percentage stand of Glabron is significantly higher than that of Comfort and Newal. On the other hand, the varieties Leiorrhynchum, Regal, Velvet and Wisconsin Barbless No. 38 do not differ significantly in this regard.

Variation due to treatment was also significant. The standard error of the difference between two 50-plot means would be $10.16\sqrt{2}/\sqrt{50}$ or 2.03%. Hence, differences of 4.06% may be considered significant. On this basis (see Table III) the varieties grown from seed receiving each of the four treatments differed significantly with regard to mean percentage stand. Hulled seed gave the highest mean stand, followed by hand-dehulled, scarified and acid-dehulled seed in the order mentioned.

The mean square for the interaction of varieties × treatments was significantly higher than the mean square for error, indicating that the varieties did not respond similarly to all treatments. Simple correlations between

the average percentage stand of the varieties of the different treatments should show the degree of any similarity in response. Correlations 15, 16 and 17 (Table VI) show the associations existing between the average percentage stands of varieties grown from hulled seed and those receiving any one of the dehulling treatments. Since the mean reduction in stand of 14% noted in the case of hulled seed is no more than would be expected from normal field germination, the values obtained have little significance in this discussion.

It will be seen that a small significant correlation exists between the average percentage stand of varieties grown from hand-dehulled and those of varieties grown from scarified seed (correlation 18), while a comparatively high correlation exists in this regard in the case of varieties grown from acid-dehulled and scarified seed (correlation 20). It would seem that certain of the factors responsible for reduction in stand when the kernels are dehulled by hand also operate when the seed is scarified. Similarly, certain mutual factors appear to be operative in reducing stand in the scarified and acid-dehulled series.

A non-significant correlation was obtained between the average percentage stands of varieties grown from hand-dehulled and those grown from acid-dehulled seed (correlation 19). This would indicate either that no mutual factors are operative in reducing stand or that the influence of any such factors are masked by the action of the sulphuric acid.

Since distorted seedlings were found in a number of the rows of all three treatments, it was thought that possibly the removal of the hull unduly predisposed the young seedlings to attack by the smut fungus at a time when they were most susceptible, and consequently resulted in their failure to emerge. Such a condition would explain the small but significant correlation existing between the average percentage stands of hand-dehulled and scarified seeds. Working on the assumption that the degree of seedling injury would be proportional to the infection percentages occurring in the mature plants, correlations were calculated between the average percentage infections and average percentage stands occurring amongst the varieties of each dehulling treatment. A non-significant correlation was found to exist between these variables in the hand-dehulled treatment (correlation 21) while a comparatively high, significant, negative value was obtained in the case of acid-dehulled seed (correlation 23) and one of doubtful significance in the case of scarified seed (correlation 22). The lack of significant correlation between percentage infection and stand of the varieties grown from hand-dehulled seed indicates that, if seedling injury from infection with *U. hordei* is a cause of reduced stand in a given variety, it is not closely associated with the susceptibility of that variety to smut, as determined by final smut percentages in mature plants.

The existence of a negative correlation between average percentage infection and stand of varieties from acid-dehulled seed suggests a direct relation between susceptibility to covered smut and kernel susceptibility to acid injury.

TABLE VI

SIMPLE CORRELATIONS OBTAINED IN THE STUDY OF PERCENTAGE COVERED SMUT INFECTION, PERCENTAGE STAND, AND NUMBER OF DAYS FROM EMERGENCE TO HEADING, OF VARIETIES GROWN FROM HULLED, HAND-DEHULLED, SCARIFIED AND ACID-DEHULLED SEED

Variables correlated	r	P
1. Smut percentages between replicates of varieties grown from hulled seed	0.476	0.02–0.01
2. Smut percentages between replicates of varieties grown from hand-dehulled seed	0.853	<0.01
3. Smut percentages between replicates of varieties grown from scarified seed	0.706	<0.01
4. Smut percentages between replicates of varieties grown from acid-dehulled seed	0.675	<0.01
5. Average smut percentages of varieties grown from hulled and hand-dehulled seed	0.522	<0.01
6. Average smut percentages of varieties grown from hulled and scarified seed	0.689	<0.01
7. Average smut percentages of varieties grown from hulled and acid-dehulled seed	0.363	0.1–0.05
8. Average smut percentages of varieties grown from hand-dehulled and scarified seed	0.748	<0.01
9. Average smut percentages of varieties grown from hand-dehulled and acid-dehulled seed	0.854	<0.01
10. Average smut percentages of varieties grown from scarified and acid-dehulled seed	0.632	<0.01
11. Percentage stand of varieties in two replicates grown from hulled seed	0.403	0.05–0.02
12. Percentage stand of varieties in two replicates grown from hand-dehulled seed	0.705	<0.01
13. Percentage stand of varieties in two replicates grown from scarified seed	0.692	<0.01
14. Percentage stand of varieties in two replicates grown from acid-dehulled seed	0.559	<0.01
15. Average percentage stand of varieties grown from hulled and hand-dehulled seed	0.466	0.02–0.01
16. Average percentage stand of varieties grown from hulled and scarified seed	0.418	0.05–0.02
17. Average percentage stand of varieties grown from hulled and acid-dehulled seed	0.286	0.2–0.1
18. Average percentage stand of varieties grown from hand-dehulled and scarified seed	0.435	0.05–0.02
19. Average percentage stand of varieties grown from hand-dehulled and acid-dehulled seed	0.159	0.5–0.4
20. Average percentage stand of varieties grown from scarified and acid-dehulled seed	0.655	<0.01
21. Average percentage infection and average percentage stand of varieties grown from hand-dehulled seed	0.107	0.6
22. Average percentage infection and average percentage stand of varieties grown from scarified seed	−0.312	0.2–0.1
23. Average percentage infection and average percentage stand of varieties grown from acid-dehulled seed	−0.734	<0.01
24. Average percentage infection (hand-dehulled) and average percentage stand (acid-dehulled)	−0.514	<0.01
25. Average percentage infection (hand-dehulled) and average percentage stand (scarified)	−0.087	0.7–0.6
26. Average number of days to heading and average percentage infection of varieties grown from hand-dehulled seed	0.542	<0.01
27. Average number of days to heading and average percentage infection of varieties grown from scarified seed	0.294	0.3–0.2
28. Average number of days to heading and average percentage infection of varieties grown from acid-dehulled seed	0.600	<0.01
29. Average number of days to heading and average percentage infection of varieties grown from hulled seed	0.169	0.5–0.4

In other words, the acid-susceptible or thin-hulled varieties appear to be more susceptible to smut. It was thought at first that this relation was apparent rather than real. That is, it was supposed that, while the acid treatment caused greater seedling mortality in the case of the thin-hulled or acid-susceptible varieties than it did in the case of the thick-hulled, it also tended to dehull the former more effectively and to induce a correspondingly higher infection. This condition also seemed to exist in the case of varieties scarified with sandpaper (correlation 22). The correlation value $r = -0.312$ obtained between average percentage stand and average percentage infection of the varieties receiving this treatment, while of doubtful significance in the light of its P value, 0.1, suggests that the thin-hulled varieties not only suffer greater seedling mortality from scarification than the thick, but also appear to be more effectively dehulled. This explanation, however, failed to hold true when it was found that a negative correlation coefficient of -0.514 also existed between average percentage infection of varieties grown from hand-dehulled seed and average percentage stand of the varieties grown from acid-dehulled seed (correlation 24). Obviously in this case the infection

TABLE VII

AVERAGE PERCENTAGE STAND AND SMUT INFECTION, AND AMOUNT OF HULL REMAINING ON KERNELS OF VARIETIES GROWN FROM ACID-DEHULLED SEED IN 1934

Variety	Number*	Amount of hull remaining over		Average percentage stand	Average percentage infection
		Embryo	Endosperm		
Lapland	N.S.N. I-32-2	very thin to absent	absent	17	32
Canadian Thorpe	C.A.N. 816	very thin to absent	absent	32	40
Hannchen	C.A.N. 837	very thin to absent	absent	41	43
Manchurian	C.A.N. 726	thin to absent	absent	15	46
Peatland	C.A.N. 722	thin to absent	absent	23	16
Shaw	C.A.N. 1047	thin to absent	absent	27	14
Bearer	C.A.N. 704	thin to absent	very thin to absent	49	28
Binder	N.S.N. I-32-8	thin to absent	very thin to absent	47	20
Gold	C.A.N. 829	thin to absent	thin to absent	33	49
Colsess	C.A.N. 772	thin to very thin	thin to absent	33	33
O.A.C. No. 21	C.A.N. 734	thin	very thin	41	4
Sol	C.A.N. 782	thin	very thin	39	4
Spartan	C.A.N. 860	moderately thick to thin	thin to absent	43	5
Swanneck	N.S.N. I-33-1	moderately thick to thin	thin to absent	41	21
Atlas	C.A.N. 702	moderately thick	thin	75	1
Comfort	C.A.N. 712	moderately thick	thin	61	6
Duckbill	C.A.N. 826	moderately thick	thin	57	4
Glabron	C.A.N. 718	moderately thick	thin	67	1
Leiorrhynchum	N.S.N. I-32-11	moderately thick	thin	68	4
Newal	C.A.N. 1089	moderately thick	thin	56	7
Regal	C.A.N. 742	moderately thick	thin	57	8
Trebi	C.A.N. 753	moderately thick	thin	63	21
Vaughn	C.A.N. 759	moderately thick	thin	57	8
Velvet	C.A.N. 755	moderately thick	thin	55	3
Wisconsin Barbless No. 38	C.A.N. 758	moderately thick	thin	60	0

* *C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

percentages obtained are entirely independent of degree of dehulling. This result shows that the relation between smut infection and kernel susceptibility to acid injury is in part a real one. Varieties showing the greatest loss of stand from acid treatment tend to be inherently more susceptible to covered smut. This conclusion does not apply in the case of varieties grown from scarified seed. A non-significant correlation was obtained between smut percentages induced by hand-dehulled seed and percentage stand resulting from scarification (correlation 25).

Further and more detailed data regarding the susceptibility of the kernels of different varieties to acid injury are given in Table VII. The varieties have been listed according to the amount of hull remaining on the kernel following treatment with sulphuric acid. The corresponding average percentage stands and smut infections are also given.

It will be noted that considerable varietal differences exist either in thickness of kernel hull or in its resistance to decomposition by acid. Varieties possessing thin hulls over the embryo after acid treatment tend to show low emergence percentages and generally high infection percentages (See Hannchen and Canadian Thorpe, Fig. 1). The opposite condition tends to prevail in the

FIG. 1. *Stand of barley varieties from seed dehulled with concentrated sulphuric acid followed by inoculation with chlamydospores of Ustilago hordei, Edmonton, 1934.*
Left to right: Hannchen, Canadian Thorpe, Leiorrhynchum, Newal, Wisconsin Barbless No. 38, Comfort, Regal, Velvet and Glabron.

case of varieties possessing moderately thick hulls after acid treatment. However, one or two exceptions occur. O.A.C. No. 21 and Sol, while showing thin hulls after acid treatment, exhibit considerable resistance to smut, which is probably physiological resistance. On the other hand, Trebi shows considerable susceptibility to smut in spite of the fact that it possesses a moderately thick or acid-resistant hull. It is interesting to note that the smooth-awned varieties all show moderately thick hulls over the embryo, after acid treatment, and these varieties produced the best stands (Fig. 1).

It would be of interest at this time to determine statistically the particular susceptibility to injury shown by certain varieties after being given the different treatments. Such a determination involves the use of cross differences. The methods involved have been clearly set out by Immer, Hayes and Powers (10).

In Table VIII are given the deviations of percentage stand of two plots of the varieties grown from seed receiving a given treatment, from the average percentage stand of the same variety grown from seed receiving the other three treatments, minus the difference between the average percentage stand of all varieties grown from seed receiving that treatment and the average percentage stand of all varieties grown from seed receiving the other three treatments. These differences express the degree of the increase in percentage stand of each variety grown from seed receiving each treatment, over the percentage stand shown by varieties grown from seed receiving the other three treatments, aside from the general increase or decrease of that treatment over the others. Plus deviations show a response more favorable and negative deviations a response less favorable than the average.

Cross differences are the differences between any two deviations in Table VIII. The error of the cross differences is calculated in the following manner: The standard error of a single total of two plots per variety per treatment

TABLE VIII

DEVIATIONS OF PERCENTAGE STANDS OF TWO PLOTS OF THE VARIETIES GROWN FROM SEED RECEIVING A GIVEN TREATMENT FROM THE AVERAGE PERCENTAGE STAND OF THE SAME VARIETY GROWN FROM SEED RECEIVING THE THREE OTHER TREATMENTS, MINUS THE DIFFERENCE BETWEEN THE AVERAGE PERCENTAGE STAND OF ALL VARIETIES GROWN FROM SEED RECEIVING THAT TREATMENT AND THE AVERAGE PERCENTAGE STAND OF ALL VARIETIES GROWN FROM SEED RECEIVING THE OTHER THREE TREATMENTS

Variety	Treatment			
	Hulled	Hand-dehulled	Scarified	Acid-dehulled
Atlas	−43.99	− 8.14	22.36	29.77
Bearer	− 6.32	− 3.81	− 6.64	20.56
Binder	− 5.99	8.53	15.02	−17.56
Canadian Thorpe	8.35	37.53	−11.98	−33.89
Comfort	− 4.32	− 4.47	−39.31	48.11
Colsess	− 6.99	12.86	23.36	−29.23
Duckbill	− 9.99	7.19	− 3.64	6.44
Glabron	− 7.32	− 7.47	− 6.31	21.11
Gold	− 1.99	32.53	0.36	−30.89
Hannchen	−22.65	30.53	27.69	−35.56
Lapland	22.01	− 7.47	− 2.31	−12.23
Leiorrhynchum	− 2.65	−81.47	37.02	47.11
Manchurian	59.35	0.53	−14.31	−45.56
Newal	24.68	−67.47	4.36	38.44
O.A.C. No. 21	3.68	30.19	−15.31	−18.56
Peatland	12.68	45.86	−12.98	−45.56
Regal	−19.32	− 0.81	− 2.31	22.44
Shaw	23.01	−11.81	10.69	−21.89
Sol	10.68	− 1.47	−22.98	13.77
Spartan	6.35	14.19	−17.98	− 2.56
Swanneck	27.68	−25.81	8.69	−10.56
Trebi	−36.32	0.86	18.02	17.44
Vaughn	−12.65	−14.14	− 0.98	27.77
Velvet	5.35	3.86	−24.31	15.11
Wisconsin Barbless No. 38	−32.65	0.53	4.36	27.77

is $10.16\sqrt{2}$ or 14.37. The standard error of the difference between one such total and the average of three others is $\sqrt{(14.37)^2 + \frac{(14.37)^2}{3}}$ or 16.58. The standard error of the difference between two such differences would be $16.58\sqrt{2}$ or 23.45. Cross differences in excess of 46.9 may be judged significant. With reference to deviations obtained from acid-dehulled seed, it will be seen the varieties Peatland, Manchurian, Hannchen, Canadian Thorpe, Gold, Colsess, Shaw and O.A.C. No. 21 appear to be particularly susceptible to acid injury. Of these, Peatland, Manchurian, Hannchen and Canadian Thorpe responded in a differential manner when compared with any one of the varieties giving plus deviations, with the exception of Duckbill. The smooth-awned varieties generally gave good plus deviations, indicating a response more favorable than the average. These data agree in a general way with those given in Table VII.

Comfort, Velvet and Sol appear to be particularly susceptible to injury from scarification. Comfort shows a differential response when compared with the varieties Atlas, Binder, Hannchen, Leiorrhynchum and Trebi. The two varieties, Leiorrhynchum and Newal showed particularly low stands from the hand-dehulled treatment. They both show differential response when compared with all varieties possessing plus deviations. It will be noted also that Duckbill responded uniformly to all treatments. O.A.C. No. 21 appears to be equally susceptible to injury from either scarification or acid-dehulling.

Effect of dehulling on earliness of heading. The varieties grown from acid-dehulled and scarified seed were found to be generally delayed in heading by 1½ and 2½ days respectively, as compared with those grown from either hulled or hand-dehulled seed. This retardation was thought to be due to slow initial growth caused by either mutilation of the embryo or loss of endosperm.

In connection with earliness of heading it is of interest to point out that a correlation value of $r = 0.542$ exists between average percentage infection and average number of days from emergence to heading in the case of the varieties grown from hand-dehulled seed (correlation 26). This indicates that the later-maturing varieties tend to be more susceptible to covered smut. A significant correlation also existed between these variables when acid-dehulled seed was used (correlation 28), but the correlation values were not significant in the cases of scarified and hulled seed (correlations 27 and 29). Low infection percentages undoubtedly explain the non-significant correlation found when hulled seed was used while the delayed development caused by scarification possibly obscured any association between date of heading and percentage smut infection.

Varietal resistance. In Table IX are given the eight-plot total and mean infection percentages of the varieties tested, together with the appropriate standard errors of the difference. The varieties are arranged in order of susceptibility. It will be seen that infection percentages range from a mean

of over 30% for the varieties Manchurian and Hannchen, to a mean of less than 1% for Atlas and Wisconsin Barbless No. 38. Lapland and Gold show mean infection percentages of over 25%; Canadian Thorpe, Colsess and Binder, mean infection percentages between 20 and 25%; and Trebi between 15 and 20%.

TABLE IX

TOTAL AND MEAN PERCENTAGE COVERED SMUT INFECTION OF EIGHT PLOTS OF EACH VARIETY

Variety	Percentage infection	
	Total	Mean
Manchurian	268	33.5
Hannchen	254	31.8
Lapland	237	29.6
Gold	220	27.5
Canadian Thorpe	196	24.5
Colsess	195	24.4
Binder	161	20.1
Trebi	145	18.1
Duckbill	117	14.6
Bearer	115	14.4
Peatland	113	14.1
Newal	106	13.3
Swanneck	82	10.3
Shaw	68	8.5
Comfort	61	7.6
Sol	54	6.8
Regal	51	6.4
Vaughn	34	4.3
Spartan	34	4.3
Glabron	24	3.0
Leirrohynchum	22	2.8
Velvet	18	2.3
O.A.C. No. 21	17	2.1
Atlas	4	0.5
Wisconsin Barbless No. 38	2	0.3
Standard error of difference	24.51	3.06

Differences between two means, exceeding twice the standard error of the difference or 6.12%, may be considered significant. It is evident that Manchurian, Hannchen, Lapland and Gold do not differ significantly in mean percentage infection. Similarly the more resistant varieties listed below Regal do not differ significantly. On the other hand, using only a few examples, Hannchen shows significantly higher mean infection percentage than Canadian Thorpe or Colsess, and Trebi a significantly higher mean infection percentage than those varieties listed below Newal.

The discussion of varietal resistance would be more clear if the varieties were classified into several commonly recognized groups. In Table III the varieties have been arranged in such a manner. The eight-plot means of each variety are given in the column to the right of the table.

Six-rowed, hulled, rough-awned types. High resistance to covered smut is shown by three varieties of this group; namely, Atlas, O.A.C. No. 21 and Vaughn. These varieties are all significantly lower in mean percentage infection than any one of the other five varieties of this group. Manchurian and Lapland evidence high susceptibility and do not differ significantly in this regard. They both show, however, significant increases over Bearer, Trebi and Peatland, varieties possessing moderate susceptibility.

Six-rowed, hulled, smooth-awned types. This group of barleys, with the exception of Newal and possibly Comfort, possesses a general resistance to covered smut. It will be noted that, while Comfort has a fairly low mean

percentage infection, it averaged 21% when grown from hand-dehulled seed. Newal shows significantly higher mean percentage smut infection than all other varieties with the exception of Comfort. Both Comfort and Regal possess significant increases over Wisconsin Barbless No. 38.

Six-rowed, hulled, hooded types. Of the three varieties comprising this group, Shaw and Sol exhibited moderate resistance while Colsess showed considerable susceptibility. Colsess significantly exceeded both Shaw and Sol with regard to mean percentage infection. Shaw and Sol did not differ significantly in this regard.

Two-rowed, hulled types. High susceptibility to covered smut is evidenced by Hannchen, Gold, Binder and Canadian Thorpe. Duckbill appeared moderately susceptible, and Spartan and Swanneck moderately resistant. Hannchen and Gold do not differ significantly with regard to mean infection percentages, but both exceed the varieties Binder, Duckbill, Swanneck and Spartan in this regard. Canadian Thorpe is exceeded significantly in mean infection percentage by Hannchen only.

The smut reactions of the varieties in the 1934 test grown from either hand-dehulled or acid-dehulled seed agreed very well with those grown from acid-dehulled seed in 1932. This conclusion is based on the reaction of 18 varieties. Values of $r = 0.811$ ($P = < .01$) and $r = 0.614$ ($P = < .01$) were obtained between the smut percentages obtained in 1932 from the Edmonton collection of smut and the average of those obtained in 1934 from hand-dehulled and acid-dehulled seed respectively. When the smut percentages obtained in 1932 from the Edmonton and Winterburn collections were averaged, the values were 0.628 ($P = < .01$) and 0.526 ($P = < .01$) respectively.

Physiologic Specialization

Literature Review

Faris (5) showed that one requirement necessary to secure high infection percentages on Hannchen and Nepal barleys, was the use of inoculum gathered from those varieties. Hannchen produced 72% of smut when inoculated with chlamydospores from Hannchen, but exhibited only from 1–2% when inoculated with smut from other sources. In a later paper (6), Faris reports the existence of five physiologic forms of covered smut based on their reactions on Nepal, Hannchen, Summit and Texas Winter barleys. Rodenhiser (14) differentiated seven physiologic forms of *U. hordei* in culture based on color, topography, surface, consistency, type of margin of colonies and chemical affinities. These forms were present in collections obtained from various localities in Minnesota, from neighboring states and foreign countries. Two of these forms, one obtained from Italy and the other from Minnesota, were shown to differ in pathogenicity. The variety Himalaya proved resistant to the Italian form and susceptible to the Minnesota form, while the variety Lion, which had hitherto shown immunity to all Minnesota smut collections, displayed susceptibility to the Italian form.

Methods and Experimental Results

An experiment was conducted at the University of Alberta in 1931, in which a test was made of the reaction of eleven barley varieties to six collections of smut. The inoculum was gathered from six points in central Alberta. Separate inoculations of all eleven varieties were made with each of the six smut collections. Seventy-five seeds were sown in duplicate ten-foot rows. The seed of the hulled varieties was not dehulled. The results of this test are summarized in Table X.

The data show that at least two of the six smut collections used are distinct physiologic forms. The collections in question are those obtained from Edmonton and Lacombe. The form from Edmonton is readily distinguished by its reaction on Eureka and Hannchen or Canadian Thorpe. Eureka showed an average of 37% infection when inoculated with the Edmonton collection, but was free from smutted plants when inoculated with any of the other five collections. Canadian Thorpe and Hannchen also proved susceptible to this collection, showing 15 and 20% average infection respectively.

TABLE X

REACTION OF ELEVEN VARIETIES OF BARLEY TO INFECTION WITH SIX COLLECTIONS OF *Ustilago hordei*, AS DETERMINED BY FIELD TESTS AT THE UNIVERSITY OF ALBERTA, EDMONTON, IN 1931

Variety	Canadian accession number	Source of inoculum and percentage of plants smutted																	
		Camrose			Edmonton*			Lacombe			Morinville			Vermilion			Wetaskiwin		
		Replicate			Replicate			Replicate			Replicate			Replicate			Replicate		
		1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
Canadian Thorpe	816	4	3	4	**20**	**10**	**15**	**20**	**24**	**22**	5	4	5	2	0	1	12	6	9
Colsess	772	3	1	2	2	2	2	10	2	6	6	12	9	2	2	2	0	5	3
Duckbill	826	0	0	0	3	1	2	3	1	2	1	1	1	0	0	0	1	0	1
Eureka	773	0	0	0	**40**	**34**	**37**	**0**	**0**	**0**	0	0	0	0	0	0	0	0	0
Glabron	718	0	1	1	0	2	1	0	0	0	0	0	0	0	2	1	0	0	0
Hannchen	837	3	1	2	**20**	**20**	**20**	6	15	11	5	6	6	6	5	6	8	4	6
O.A.C. No. 21	734	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peatland	722	13	6	10	2	8	5	9	8	9	5	6	6	1	5	3	8	1	5
Regal	742	2	1	2	1	0	1	1	4	3	1	1	1	1	3	2	1	0	1
Spartan	860	1	0	1	0	0	0	1	3	2	1	1	1	0	4	2	2	1	2
Trebi	753	3	11	7	5	3	4	6	4	5	0	3	2	1	1	1	2	3	3

**This collection of smut was obtained from the variety Success, grown at the University of Alberta.*

The physiologic form represented by the collection of smut from Lacombe is easily distinguished from the Edmonton form by its failure to infect the variety Eureka and the comparatively high percentage of smut it produced on Canadian Thorpe. The collections from Camrose, Morinville, Vermilion and Wetaskiwin do not appear to be sufficiently different from the Lacombe collection to be considered as separate forms. The Vermilion collection produced a low percentage of smutted plants on Canadian Thorpe in contrast

to the Lacombe collection, but this may be due to a lower degree of spore viability. This is indicated by the low percentage of plants infected in all the varieties tested with this collection.

The reliability of the data obtained in this experiment is well illustrated in the high degree of correlation between the infection percentages of the different varieties in the two replicates. The correlation coefficient between the percentage infection in the two replicates of the 11 varieties with the six collections of smut is $+ 0.832$ ($P = <0.01$). In many instances there was no infection on either replicate. When the correlation coefficient is calculated, using the infection percentages from only those plots in which there was some infection, it is $+0.870$ ($P = <0.01$).

It will be noted in Table X that no smutted plants appeared in any of the rows of O.A.C. No. 21. Regal, Duckbill, Glabron and Spartan appeared highly resistant to all the collections of smut used in this test.

Discussion

There is an urgent need for improved methods for the testing of barley varietal reaction to the covered smut disease. The use of hulled seed has not proved satisfactory, several workers having reported failure to differentiate between susceptible and resistant sorts owing to the low infection persentages obtained (1, 2, 14, 15). Dehulling of the kernels prior to inoculation has resulted in increased infections (1, 6, 17). However, no really satisfactory method of dehulling has yet been reported. Dehulling by hand is laborious and impracticable when large populations are involved. The use of sulphuric acid as suggested by Briggs (1) did not prove altogether satisfactory when used in the concentrated form, by one of the authors, to dehull kernel lots of hybrid material (11). However, the possibilities of this chemical have by no means been exhausted. The feasibility of scarification or some other type of mechanical injury of the seed coat as a means of dehulling has received little attention.

From the point of view of the plant breeder, methods of dehulling that will give reliable and high infections consistent with ease of application are desirable. In varietal testing where only comparative reaction is desired, it is not imperative to have methods giving the highest infections provided that the methods adopted induce infections sufficiently high to allow of the separation of resistant and susceptible sorts.

From the results of the present study, it is evident that the infection percentages obtained from hulled seed are too low and too unreliable to be of value in testing varietal reaction to the covered smut disease. Dehulling by hand, acid or scarification gave significant increases in smut infection. Highest and most reliable infection percentages were obtained from hand-dehulled seed. However, this method involved the greatest time and labor. Acid-dehulled seed gave higher infection percentages than scarified seed, but exhibited considerably more seedling injury as shown by reduced stands.

Acid-dehulling involved the least time and labor of the three methods tested. It must be borne in mind, however, that since seed free from mechanical injury is essential for the success of this treatment, considerable work of a preliminary nature may be necessary in selecting sound seed. The greatest criticism which may be directed at sulphuric acid as a dehulling agent is the seedling injury resulting from its use in the case of certain varieties, which tends to detract somewhat from the significance of the results obtained. Increasing the number of dehulled seeds for the test to at least 100 should increase the reliability of the results obtained by this method.

Scarified seed gave stands only slightly more reduced than those from hand-dehulled seed. However, the agreement of infection percentages induced by scarification with those induced by hand-dehulling was poor in the case of certain varieties. Bearer and Comfort gave 26 and 21% smut infection respectively when grown from hand-dehulled seed and only three and two per cent when grown from scarified seed. Similarly, Hannchen and Gold showed considerably less susceptibility when grown from scarified rather than from hand-dehulled seed. As good agreement exists between replicates, it would appear that scarification as practised in this investigation is ineffective in inducing infection within certain varieties. Generally, however, this method differentiated fairly well between susceptible and resistant varieties. It is felt that, with further investigation, scarification of barley seed as a means of inducing smut infection will prove to be of considerable value in the testing for varietal reaction.

It appears evident, from the data presented, that hand-dehulling should be practised when maximum infections are desired. Removal of the complete hull is not necessary. In the present work only the embryo was exposed and high infection percentages were obtained.

References

1. Briggs, F. N. Dehulling barley seed with sulphuric acid to induce infection with covered smut. J. Agr. Research, 35 : 907-914. 1927.
2. Conners, I. L. Smut investigations. Report of the Dominion Botanist for the year 1926, p. 115. Dept. Agr., Dominion of Canada. 1927.
3. Conners, I. L. Reports of the Canadian Plant Disease Survey for the years 1929, 1931, 1932 and 1933.
4. Conners, I. L. and Eardley, E. A. Report of the Canadian Plant Disease Survey for the year 1930.
5. Faris, J. A. Factors influencing infection of *Hordeum sativum* by *Ustilago hordei*. Am. J. Botany, 11 : 189-214. 1924.
6. Faris, J. A. Physiological specialization of *Ustilago hordei*. Phytopathology, 14 : 537-557. 1924.
7. Fisher, R. A. Statistical methods for research workers. Edinburgh: Oliver and Boyd, London. 2nd ed. 1928.
8. Güssow, H. T. and Conners, I. L. Studies in cereal diseases. I. Smut diseases of cultivated plants, their cause and control. Dom. Canada, Dept. Agr. Bull. 81, n.s. 1927.
9. Hanna, W. F. and Popp, W. Reaction of barley varieties to covered smut. Report of Dominion Botanist for the year 1930, p. 72. Dept. Agr., Dominion of Canada. 1931.

10. Immer, F. R., Hayes, H. K. and Powers, L. Statistical determination of barley varietal adaptation. J. Am. Soc. Agron. 26 : 403-419. 1934.

11. Johnston, W. H. Studies on the dehulling of barley kernels with sulphuric acid and on the inheritance of reaction to covered smut *Ustilago hordei* (Pers.) K. & S. infection in crosses between Glabron and Trebi barleys. Can. J. Research, 11 : 458-473. 1934.

12. Leukel, R. W. Seed treatment for controlling covered smut of barley. U.S. Dept. Agr. Tech. Bull. 207. 1930.

13. McCurry, J. B. Report of the Canadian Plant Disease Survey for the years 1927-28.

14. Rodenhiser, H. A. Physiologic specialization in some cereal smuts. Phytopathology, 18 : 955-1003. 1928.

15. Stakman, E. C. Diseases of cereal and forage crops in the United States in 1921. U.S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. sup. 21 : 208-209. 1922. (Mimeograph).

16. Taylor, J. W. and Zehner, M. G. Effect of depth of seeding on the occurrence of loose and covered smut in winter barley. J. Am. Soc. Agron. 23 : 132-141. 1931.

17. Tisdale, W. H. An effective method of inoculating barley with covered smut. Phytopathology, 13 : 551-554. 1923.

A COMPARISON OF VARIOUS HARVESTING METHODS IN RESPECT TO MOISTURE CONTENT AND GRADE OF THE GRAIN[1]

(FINAL REPORT)

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Abstract

In a moisture and grade survey of grain harvested by various methods in parts of Manitoba and Saskatchewan in 1933, it was found that straight-combined wheat showed a much greater percentage of tough and damp samples than either stook-threshed or swath-combined samples. Of 246 straight-combined samples, 18% were tough and 7% damp; of 212 stook-threshed samples, 10% were tough and none damp; of 184 swath-combined samples 6.5% were tough and 0.5% were damp. These results confirm those obtained in 1932.

In common wheat there was a marked decrease in grade between the early- and late-season samples. Average grades were 1.37, 1.32 and 1.28 for stook-threshed, straight-combined and swath-combined samples respectively. This is quite the reverse of the order found in 1932. Taking the two years' samples, collectively, the average grades are 1.18, 1.28 and 1.36 for the methods in the order given above. In view of the conflicting results for the two consecutive seasons, no definite conclusion can be drawn with regard to the average grade of common wheat threshed by these three methods.

With durum wheat in 1933 the average grades were 1.29, 1.79 and 2.00 for stook-threshed, straight-combined and swath-combined samples respectively. Differentiation of the three harvesting methods on basis of grade of durum wheat was greater in 1933 than in 1932. This points firmly to the conclusion that stook threshing is the best method for this class of wheat in Manitoba.

A small series of 32 barley samples collected in Manitoba in 1933 showed no differentiation in moisture content as a result of method of harvesting. However, stook-threshed samples of barley tended to grade higher than those threshed by either of the combine methods.

In an earlier paper Larmour, Geddes and Cameron (1) discussed the conditions which led to the investigation of variation in moisture and grade of grain harvested in different ways. While the survey of 1932 was fairly comprehensive in covering the general areas of Western Canada in which combine harvesting is practised, it was considered advisable to conduct a similar survey in the following season in order to avoid the dangers in conclusion that are liable to be made when depending on only one year's data. This paper is a report of the observations on samples collected in 1933 and a general summary of the results of the whole project.

Owing to the conditions of extreme drought prevailing in 1933 in central and southern Saskatchewan and Alberta, the number of samples was reduced considerably. No field samples were collected in Alberta because practically all the normal combine area in that province was severely affected by drought.

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In Saskatchewan, the areas of greatest combine concentration, namely the Rosetown-Kindersley district, had almost a complete failure, but the crop was fairly good in the middle-south of the province and most of the sampling in 1933 was done in this area. In Manitoba the area covered was approximately the same as in the preceding year. Collection of samples and determination of moisture and grades were carried out as in 1932.

As in the previous work (1) the collections were divided into three parts: (*i*) the early harvest season, before any rain had occurred, (*ii*) a short period after one general rain and (*iii*) the later harvest season after two or more rains had occurred. The numbers of samples collected in these periods are given in Table I.

TABLE I

CLASSIFICATION OF SAMPLES ACCORDING TO METHOD OF HARVESTING, EXPOSURE TO RAINFALL AND ORIGIN, 1933

	Before rain		After one rain		After two or more rains		Grand total	
	No.	%	No.	%	No.	%	No.	%
Stook-threshed—								
Manitoba common	61	9.5	17	2.6	9	1.4	87	13.6
Manitoba durum	15	2.3	6	0.9	43	6.7	64	10.0
Saskatchewan	10	1.6	4	0.6	47	7.3	61	9.5
Total	86	13.4	27	4.1	99	15.4	212	33.1
Straight-combined—								
Manitoba common	20	3.1	2	0.3	–	–	22	3.4
Manitoba durum	15	2.3	2	0.3	13	2.0	30	4.7
Saskatchewan	135	21.0	13	2.0	46	7.2	194	30.2
Total	170	26.4	17	2.6	59	9.2	246	38.3
Swath-combined—								
Manitoba common	47	7.3	14	2.2	7	1.1	68	10.6
Manitoba durum	28	4.4	6	0.9	44	6.8	78	12.1
Saskatchewan	17	2.6	2	0.3	19	3.0	38	5.9
Total	92	14.3	22	3.4	70	10.9	184	28.6
Grand totals, all methods	348	54.2	66	10.3	228	35.5	642	100.0

The total number of samples collected was 642 as compared with 1028 in the 1932 season. Of these 33% were stook-threshed, 38% straight-combined and 29% swath-combined: 54% were collected before any rain, 10% after one rain and 36% after two or more rains.

Moisture in Wheat Samples Harvested Before Rain

A summary of the distribution of moisture in this class is given in Table II. All the stook-threshed samples were dry; of the 170 straight combined samples, 14% were tough and 8% were damp; of the 82 swath-combined samples 4% were tough and all the rest dry. These results confirm those of the preceding

TABLE II

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED BEFORE ANY RAIN, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	27	4	31	36.1	10.4	3	30	33	19.4	10.4	26	8	34	37.0	10.2
10.9–11.7	18	3	21	24.4	11.2	9	28	37	21.8	11.3	18	5	23	25.0	11.2
11.8–12.6	16	2	18	20.9	12.1	9	20	29	17.1	12.1	13	1	14	15.2	12.2
12.7–13.5	10	1	11	12.8	12.9	6	18	24	14.1	13.0	10	3	13	14.1	13.0
13.6–14.4	5	—	5	5.8	14.0	0	9	9	5.3	14.0	4	—	4	4.3	14.0
Total			86	100.0				132	77.7				88	95.6	
Tough—															
14.5–15.3	—	—	—	—	—	2	8	10	5.9	15.0	3	—	3	3.3	14.8
15.4–16.2	—	—	—	—	—	—	2	2	1.2	15.9	1	—	1	1.1	15.8
16.3–17.0	—	—	—	—	—	3	9	12	7.0	16.6	—	—	—	—	—
Total								24	14.1				4	4.4	
Damp—															
17.1–17.9	—	—	—	—	—	—	6	6	3.5	17.6	—	—	—	—	—
18.0–18.8	—	—	—	—	—	3	1	4	2.4	18.4	—	—	—	—	—
> 18.9	—	—	—	—	—	—	4	4	2.3	22.1	—	—	—	—	—
Total								14	8.2						
Total in each group			86	100.0				170	100.0				92	100.0	
Total in series								348							

year, when in a similar group 98% of both the stook-threshed and swath-combined samples were below 14.5% moisture, while the straight combined samples showed 16% tough and damp compared with 22% for 1933. Again it must be concluded that even under the most favorable harvesting conditions at the beginning of the season, there is a tendency for straight-combine operators to start harvesting too early.

Samples Collected After One Rain

This group was relatively larger than in the previous season, consisting of 66 samples or 10% of the total, as compared with 77 or 7.5% of the total in 1932. The data in Table III show that 15% of the stook-threshed, 12% of the straight-combined and none of the swath-combined samples collected in this period were tough; none were damp. This is quite different from the observations for the corresponding period of 1932 when these three methods in the order named gave 13%, 32% and 75% tough and damp samples.

Samples Collected after Two or More Rains

The number of samples in this class in 1933 was 35.5% of the total as compared with 50% for the preceding year. The fall rains were, in general, later in 1933 than in 1932. The grain, therefore, had every chance to ripen and consequently very few of the tough and damp samples could be attributed to immature grain. The data in Table IV show that of the stook-threshed samples 17.2% were tough and none damp; in the corresponding group of 1932 only 3% were tough. The straight-combine method gave 32.2% tough and 5.1% damp; in the previous season there were 25% tough and 3% damp. The swath-combine method showed 11.4% tough and 1.4% damp, compared with 9% and 2% respectively in 1932. On the whole, there was a greater incidence of tough and damp samples in 1933 than in 1932 during the late harvesting period.

The data of Tables II, III and IV are combined in Table V, summarizing the distribution of moisture for all samples collected in 1933. Of 212 stook-threshed samples 10% were tough and none damp; of 246 straight-combined samples 18% were tough and 7% damp; of 184 swath-combined samples 6.5% were tough and 0.5% damp.

Close examination of Table V shows that the degree of toughness did not vary much with the three harvesting methods. The weighted average moistures of the tough samples are 15.4%, 15.5% and 15.3% for stook-threshed, straight-combined and swath-combined respectively. The dampest samples were obtained by the straight-combine method.. Of the 18 damp samples in 1933, 17 were straight combined and of these 5 were above 19.7% moisture and averaged 22.1% moisture content. In the straight grade moisture range, the stook-threshed and straight-combined samples show about the same average moistures, while the swath-combined samples tend to be somewhat lower in moisture.

TABLE III

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED AFTER ONE RAIN, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	6	—	6	22.2	10.4	—	1	1	5.9	10.6	5	—	5	22.7	10.0
10.9–11.7	8	—	8	29.6	11.4	1	2	3	17.6	11.5	7	—	7	31.8	11.2
11.8–12.6	6	1	7	25.9	12.1	—	5	5	29.4	12.3	7	2	9	40.9	12.0
12.7–13.5	—	—	—	—	—	2	3	5	29.4	13.2	1	—	1	4.6	13.5
13.6–14.4	1	1	2	7.4	13.7	1	—	1	5.9	14.4	—	—	—	—	—
Total			23	85.2				15	88.2				22	100.0	
Tough—															
14.5–15.3	1	2	3	11.1	14.8	—	—	—	—	—	—	—	—	—	—
15.4–16.2	1	—	1	3.7	16.2	—	2	2	11.8	15.6	—	—	—	—	—
16.3–17.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total			4	14.8				2	11.8						
Damp—															
17.1–17.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18.0–18.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
> 18.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total in each group			27	100.0				17	100.0				22	100.0	
Total in series	66														

TABLE IV

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED AFTER TWO OR MORE RAINS, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	—	—	—	—	—	1	—	1	1.7	10.7	4	—	4	5.7	10.6
10.9–11.7	10	1	11	11.1	11.4	—	2	2	3.4	11.5	13	1	14	20.0	11.4
11.8–12.6	15	5	20	20.2	12.1	2	2	4	6.8	12.4	13	5	18	25.7	12.1
12.7–13.5	16	14	30	30.3	13.1	4	8	12	20.3	13.0	13	7	20	28.6	13.0
13.6–14.4	8	13	21	21.2	14.0	3	15	18	30.5	14.0	1	4	5	7.1	14.0
Total			82	82.8				37	62.7				61	87.1	
Tough—															
14.5–15.3	3	6	9	9.1	14.8	1	11	12	20.3	14.8	1	1	2	2.9	14.6
15.4–16.2	—	5	5	5.1	15.9	1	5	6	10.2	15.8	4	1	5	7.1	15.6
16.3–17.0	—	3	3	3.0	16.6	—	1	1	1.7	16.6	1	—	1	1.4	16.3
Total			17	17.2				19	32.2				8	11.4	
Damp—															
17.1–17.9	—	—	—	—	—	—	2	2	3.4	17.2	—	—	—	—	—
18.0–18.8	—	—	—	—	—	—	—	—	—	—	1	—	1	1.4	18.1
> 18.8	—	—	—	—	—	1	—	1	1.7	19.9	—	—	—	—	—
Total								3	5.1				1	1.4	
Total in each group			99	100.0				59	100.0				70	100.0	
Total in series	228														

TABLE V

DISTRIBUTION OF MOISTURE, ALL WHEAT SAMPLES COLLECTED IN 1933

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	33	4	37	17.5	10.4	4	31	35	14.2	10.4	35	8	43	23.4	10.2
10.9–11.7	36	4	40	18.9	11.3	10	32	42	17.1	11.3	38	6	44	23.9	11.3
11.8–12.6	37	8	45	21.2	12.1	11	27	38	15.4	12.2	33	8	41	22.3	12.1
12.7–13.5	26	15	41	19.3	13.0	12	29	41	16.7	13.0	24	10	34	18.5	13.0
13.6–14.4	14	14	28	13.2	14.0	4	24	28	11.4	14.0	5	4	9	4.9	14.0
Totals			191	90.1				184	74.8				171	93.0	
Tough—															
14.5–15.3	4	8	12	5.7	14.8	3	19	22	8.9	14.9	4	1	5	2.7	14.7
15.4–16.2	1	5	6	2.8	16.0	1	9	10	4.1	15.8	5	1	6	3.3	15.6
16.3–17.0	—	3	3	1.4	16.6	3	10	13	5.3	16.6	1	—	1	0.5	16.3
Totals			21	9.9				45	18.3				12	6.5	
Damp—															
17.1–17.9	—	—	—	—	—	—	8	8	3.2	17.5	—	—	—	—	—
18.0–18.8	—	—	—	—	—	3	1	4	1.6	18.4	—	—	—	—	—
18.9–19.7	—	—	—	—	—	—	—	—	—	—	1	—	1	0.3	19.1
> 19.7	—	—	—	—	—	1	4	5	2.0	22.1	—	—	—	—	—
Totals								17	6.9				1	0.5	
Grand totals			212	100.0				246	100.0				184	100.0	
Total in series								642							

Comparison of Results of 1932 and 1933

A summary of the data obtained in 1932 and 1933 and of the combined data for the two years is given in Table VI, in which the percentages of samples higher than 14.4% in moisture content are shown. The greatest variations are found in the straight-combined samples in the three groupings, "before

TABLE VI

PERCENTAGE OF SAMPLES HIGHER THAN 14.4% IN MOISTURE CONTENT IN THE 1932, 1933 AND COMBINED COLLECTIONS

	Before rain, %	After one rain, %	After two or more rains, %	All samples, %
Stook-threshed—				
1932	3	13	3	3
1933	0	15	17	10
Collectively	1	14	8.4	5.4
Straight-combined—				
1932	19	34	28	25
1933	38	12	37	25
Collectively	19.5	28	30	25
Swath-combined—				
1932	2	75*	11	9
1933	4	0	13	7
Collectively	3	20	12.4	8

*This percentage was based on only 8 samples.

rain", "after one rain" and "after two or more rains". These differences disappear in the results for the whole season; the 1932 and 1933 collections each showed 25% tough and damp samples by this method. The swath-combined samples had 9% and 7% tough and damp for 1932 and 1933 respectively, with 8% for the two years combined. The greatest difference between the two years occurred in the stook-threshed samples, in which there were 3% and 10% tough and damp for 1932 and 1933 respectively. This was due to the high percentage of tough samples in the late season collection in 1933.

The Relation of Harvesting Method to the Grade of the Sample

Common Wheat

The official grades of all common wheat samples collected in 1933 are given in Table VII. The average grade was calculated as described by Larmour, Geddes and Cameron (1) by assigning the arbitrary values, 0, 1, 2, 3, 4, 5, 3, 4, 5 and 6 to grades 1 Hard, Nos. 1, 2, 3 and 4 Northern, No. 5, No. 1 Nor. rejected, No. 2 Nor. rejected, No. 3 Nor. rejected and No. 4 Nor. rejected, respectively. Thus the smaller the figure, the higher is the average grade.

TABLE VII

DISTRIBUTION OF SAMPLES ACCORDING TO GRADE—COMMON WHEAT 1933

Grade	Stook-threshed				Straight-combined				Swath-combined			
	Man.	Sask.	Total	%	Man	Sask.	Total	%	Man.	Sask.	Total	%
Samples collected before rain												
1 Hard	6	3	9	12.7	—	26	26	16.8	3	11	14	21.9
1 Northern	46	5	51	71.8	16	68	84	54.2	34	3	37	57.8
2 Northern	9	1	10	14.1	3	33	36	23.2	7	3	10	15.6
3 Northern	—	—	—	—	1	6	7	4.5	3	—	3	4.7
4 Northern	—	—	—	—	—	1(1)	1	0.7	—	—	—	—
No. 5	—	1	1	1.4	—	1(1)	1	0.7	—	—	—	—
Total	61	10	71		20	135	155		47	17	64	
Average grade	1.07				1.20				1.00			
Samples collected after one rain												
1 Hard	2	1	3	14.3	—	1	1	6.7	—	—	—	—
1 Northern	13	1	14	66.7	2	11	13	86.7	6	1	7	43.8
2 Northern	2	1	3	14.3	—	1	1	6.7	8	1	9	56.2
3 Northern	—	1	1	4.7	—	—	—	—	—	—	—	—
Total	17	4	21		2	13	15		14	2	16	
Average grade	1.10				1.0				1.56			
Samples collected after two rains												
1 Hard	—	1	1	1.8	—	—	—	—	—	—	—	—
1 Northern	4	13	17	30.9	—	10	10	21.7	1	2	3	11.3
2 Northern	5	23	28	50.9	—	34	34	73.9	1	16	17	65.4
3 Northern	—	7	7	12.7	—	2	2	4.4	5	1	6	23.1
4 Northern	—	1	1	1.8	—	—	—	—	—	—	—	—
No. 5	—	1(2)	1	1.8	—	—	—	—	—	—	—	—
Total	9	46	55			46	46		7	19	26	
Average grade	1.91				1.83				2.11			

(1) *Rejected, mouldy.* (2) *Rejected.*

In the group of samples collected early in the harvest season before any rain had fallen, the average grades were 1.07, 1.20, 1.00 for stook-threshed, straight-combined and swath-combined samples respectively. The lower average grade of the straight-combined samples probably has no relation to the method of harvesting in this instance, but is associated with the fact that most of them were collected from the dry area of south-central Saskatchewan. The lower average grade, therefore, was attributable mainly to low weight per bushel.

The group representing collections after the first rain showed a slight decrease in average grade of the stook-threshed, an increase from 1.2 to 1.0 in the straight-combined and a marked decrease in average grade in the swath-combined, 1.0 to 1.56. As the number in this group was small, not much significance can be attached to these changes.

The samples collected in the latter part of the harvest season showed a very distinct lowering of grade with all methods of harvesting. Compared with the early harvest samples, the grade lowering was 0.84, 0.63 and 1.11 for the stook-threshed, straight-combined and swath-combined samples respectively.

Comparison of average grades for the various groups in 1932, 1933 and for the two years collectively can be made by means of the summary given in Table VIII. The grades for the 1933 stook-threshed and straight-combined samples were lower than those of 1932, but the 1933 swath-combined samples graded higher than those of 1932. Considering both years' samples collectively, it is evident that under early season conditions, before rains occur, the swath-combine method yields the best grade and the straight-combine method the lowest grade, the average values being 0.86, 0.93 and

TABLE VIII

AVERAGE GRADES FOR COMMON WHEAT IN 1932, 1933 AND IN THE TWO YEARS COLLECTIVELY

	Average grades			
	Before rain	After one rain	After two or more rains	All samples
Stook-threshed—				
1932	0.87	0.60	1.20	1.04
1933	1.07	1.10	1.91	1.37
Collectively	0.93	1.00	1.52	1.18
Straight-combined—				
1932	0.96	0.80	1.80	1.24
1933	1.20	1.00	1.83	1.32
Collectively	1.14	0.84	1.80	1.28
Swath-combined—				
1932	0.60	0.50	2.27	1.40
1933	1.00	1.56	2.11	1.28
Collectively	0.86	1.44	2.21	1.36

1.14 for swather, stook-threshed and straight-combine methods. On the other hand, during the latter part of the harvest season, after several rains have occurred, the order is changed and stook-threshing yields the best grade and the swath-combining the poorest, the average values being 1.52, 1.80 and 2.21 for stook-threshing, straight-combining and swath-combining respectively.

Durum Wheat

In 1933 there were collected in Manitoba 172 durum wheat samples. These were considered with the common wheat in the discussions of moisture, but must be considered as a separate group for discussion of grade. A summary of the distribution in the grades and the average grades is given in

TABLE IX

SUMMARY SHOWING THE GRADES OF DURUM WHEAT SAMPLES COLLECTED IN 1933

Method of harvesting	No. of samples	% of samples grading				Average grade
		1 A.D.	2 A.D.	3 A.D.	4 A.D.	
Stook-threshed—						
Before rain	15	80.0	20.0	–	–	1.20
After one rain	6	66.6	33.4	–	–	1.33
After two rains	43	72.1	27.9	–	–	1.28
						Mean 1.29
Straight-combined—						
Before rain	15	46.7	26.7	26.7	–	1.87
After one rain	2	100	–	–	–	1.00
After two rains	13	38.5	38.5	23.0	–	1.83
						Mean 1.79
Swath-combined—						
Before rain	28	60.7	39.3	–	–	1.39
After one rain	6	83.3	16.7	–	–	1.17
After two rains	44	11.4	34.1	50.0	4.5	2.48
						Mean 2.00

Table IX. For computing average grades of durum wheat the arbitrary values 1, 2, 3 and 4 were assigned to the grades 1 A.D.*, 2 A.D., 3 A.D. and 4 A.D. respectively.

Differences in average grade between samples collected early and late in the harvesting season were small in the stook-threshed and straight-combined grain, but relatively large in the swath-combined grain. Average grades for the three methods in the above order were 1.29, 1.79 and 2.00 respectively, showing that the straight-combined samples were, on the average, one half grade and the swath-combined samples three-quarters of a grade lower than the stook-threshed samples. These results are somewhat different from those

*Amber durum.

obtained in the previous season when it was found that the stook-threshed and straight-combined samples were not appreciably different in average grade and the swath-combined samples were only lower by about one-seventh of a grade.

The average grades were lower in 1933 than in 1932 for the samples collected, as can be seen from the comparative values given in Table X.

TABLE X

SUMMARY OF THE AVERAGE GRADES OF DURUM WHEAT SAMPLES FOR 1932, 1933 AND FOR THE TWO YEARS COLLECTIVELY

Method and year	Before rain	After one rain	After two or more rains	All samples
Stook-threshed—				
1932	1.02	1.10	1.55	1.25
1933	1.20	1.33	1.28	1.29
Collectively	1.06	1.19	1.41	1.26
Straight-combined—				
1932	1.11	1.00	1.50	1.23
1933	1.87	1.00	1.83	1.79
Collectively	1.45	1.00	1.64	1.45
Swath-combined—				
1932	1.01	1.80	2.10	1.40
1933	1.39	1.17	2.48	2.00
Collectively	1.13	1.42	2.32	1.67

Relation of Harvesting Method to Moisture and Grade of Barley in 1933

In 1933 a number of barley samples were collected and although these are too few to afford reliable conclusions, they are being included as a matter of record and to give some indication of what might be expected of the three harvesting methods. Since there were only 32 samples altogether, no attempt has been made to divide them into groups according to weather, as was done in the case of wheat. It might be noted, however, that 23 of the 32 samples were collected during the period August 8-11, inclusive. The results are given in Table XI.

TABLE XI

SUMMARY OF OBSERVATIONS ON BARLEY SAMPLES COLLECTED IN 1933

Method of harvesting	No. of samples grading					Total	Average grade*	Average moisture, %
	3 Ex. C.W.	3 C.W.	4 C.W.	5 C.W.	6 C.W.			
Stook-threshed	5	5	1	–	–	11	3.6	10.7
Straight-combined	1	3	1	–	1	6	4.5	10.6
Swath-combined	4	7	1	2	1	15	4.3	10.6

**In computing average grades, the empirical values 3, 4, 5, 6, 7 were assigned to the grades 3 Ex. C.W., 3 C.W., 4 C.W., 5 C.W. and 6 C.W. respectively.*

No tough or damp samples were obtained by any of the harvesting methods. The average moistures were 10.7%, 10.6% and 10.6% for stook-threshed, straight-combined and swath-combined samples respectively, indicating no difference as a result of the methods. With respect to grades, the average values were 3.6, 4.5 and 4.3 for the three methods in the order named above. The stook-threshed samples were 0.7 to 0.9 grades higher than those obtained by the other two methods. There was little difference in grade between the straight-combined and swath-combined samples. Summing up the observations on barley it may be said that with 32 samples collected in 1933 harvesting method made no difference in moisture content and that the stook-threshed samples were, on the average, about three-quarters of a grade better than those threshed by the other two methods.

Conclusions

The 1933 study of the three methods of harvesting wheat, namely, stook-threshing, straight-combining and swath-combining, confirmed the conclusions drawn from the study of the previous year in respect to moisture content of the threshed grain. In each year it was found that 25% of all straight-combined wheat samples were tough or damp. With swath-combining there were 9% and 7% in 1932 and 1933 respectively, or 8% of the collective samples of both years. Stook-threshing showed only 3% and 10% respectively or 5.4% for the collective samples of both years. It must not be inferred that these percentages are thought to represent the whole crop in these years. Particularly in the case of the stook-threshed samples, it should be kept in mind that these were collected in the combine areas to serve as a means for comparison. They represent, therefore, only the more southerly areas where the grain tends to mature early. In either of these years, samples from the northerly areas would doubtless have shown incidence of tough and damp samples in excess of 5.4%.

In both 1932 and 1933, the harvest season in the middle and southern parts of Western Canada, where the combining method is used, was particularly favorable inasmuch as the crop ripened rapidly and quite uniformly and weed growth was not excessive. With 25% of the straight-combined wheat turning out tough and damp under such conditions, it is likely that the percentage would increase considerably in a poor harvest season. The reason for this high percentage of tough and damp wheat seems to be that the wheat straw becomes brittle enough for good threshing before the grain itself is dried to below 14.4% moisture, and the operators, fearing damage to the standing grain, become overanxious and start harvesting too early.

Reference

1. Larmour, R. K., Geddes, W. F. and Cameron, D. Can. J. Research, 9 : 486-501. 1933

A STUDY OF THE RESPIRATION AND HEATING OF DAMP WHEAT[1]

BY R. K. LARMOUR[2], J. S. CLAYTON[3] AND C. L. WRENSHALL[3]

Abstract

Respiration and heating studies were made on hard red spring wheat.

Estimation of the true respiration of hard red spring wheat is complicated by the respiration of fungi which develop on damp wheat. The germination and growth of fungi can be controlled effectively by toluene or carbon tetrachloride vapor. In the presence of vapor of these substances carbon dioxide production goes on at a low rate and no heating occurs in wheat of 25% moisture content. The odor of the vapor disappears in the course of air-drying.

Exposure of damp wheat to carbon tetrachloride for 25 days produced no deleterious effect on the quality.

The problem of storage and transportation of damp wheat assumes grave proportions in years when protracted rainfall occurs during the latter part of the harvest season. Neither farmers nor the country elevators have means for drying wheat and it must therefore be shipped with dispatch to the terminal elevators where it can be dried to a moisture content suitable for storage. When there is much damp wheat in the country, heavy losses from heating occur in storage in farmers' bins, in country elevator bins and in transit. There is no remedy for bin-burned or heated wheat; it is irreparably damaged and is fit only for feed. It cannot be blended with sound wheat even in small amounts because the moldy odor is very persistent and carries through into the flour.

In normal seasons the small amount of damp wheat that comes on the market can be easily handled either by rushing it rapidly to the driers at the terminals or by mixing it with normal dry wheat. In the latter case distribution of moisture takes place rapidly and if the mixing is done skilfully, there is little danger of heating.

With the advent of the combine harvester, a new factor was introduced, inasmuch as there seemed to be a tendency on the part of the operators to cut the grain too early. This is quite natural, as early cutting reduces the risk of damage by bleaching and loss by shelling. Larmour, Geddes and Cameron (7) in an extensive survey of harvesting methods showed that combined wheat, on the average, tends to be higher in moisture than stook-threshed wheat, but the amount of damp wheat occurring as a result of combine harvesting should present no serious handling problem in normal years. On several occasions, however, statements have been made to the

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effect that combined wheat of apparently normal moisture content tends to heat more readily than stook-threshed wheat and a number of cases of spoilage in transit have been reported. To account for this the theory has been advanced that the combined wheat, having had no chance to "sweat" before harvesting, undergoes this process in the bin or car and this provides conditions favorable for heating. In the older method of harvesting "sweating" occurs in the stook, where the small bulk and the comparatively great aeration precludes the danger of heating. It seems probable that in the course of desiccation of wheat during the latter part of the ripening process there is a stage at which there occurs a redistribution of bound and free water in the grain, accompanied by a syneretic effect which makes the grain feel damp, even though there has been no absolute change in the moisture content. Unless it is assumed that some very fundamental change in the wheat berry takes place in the course of sweating, this phenomenon alone would scarcely be sufficient to explain the observations referred to above.

The respiration and heating of cereal grains has attracted the attention of many workers on account of the practical significance of the relations of moisture content, heating and the keeping quality of the stored grain. Among the early workers Kolkwitz (6) using barley, and Quam (14) using oats, showed that the rate of carbon dioxide production increased with increasing moisture content after a certain value of the latter had been attained. Bailey and Gurjar (1), using wheat, showed that respiration depended upon the following variable factors: moisture content, temperature, concentration of carbon dioxide, oxygen supply, period of dampness, and also was partially governed by inherent factors in the wheat itself, such as protein content, plumpness and frost damage.

The role of micro-organisms in heating of stored organic material was pointed out as early as 1907 by Miehe (9), who observed their presence in heating hay. Peirce (13) and later Darsie, Elliott and Peirce (3) showed that micro-organisms had much to do with rise in temperature of stored seeds. More recently the investigations of Gilman and Barron (4), Miehe (10), Norman (11), Bakke and Noecker (2), Isatschenko *et al.* (5) and Swanson (17) have shown definitely that heating of damp grain and other organic substances such as hay and straw is usually accompanied by growth of fungi. While the heat produced in stored damp grain is doubtless due to both respiration of the embryo and the growth of fungi, several of the aforementioned workers seem to favor the suggestion that the micro-organisms are mainly responsible for the high temperatures which result in bin-burning. To date there has not been reported any very successful segregation of these two factors, the difficulty in this connection being to sterilize the grain without injuring the embryo.

In the investigation herein reported it was found possible to prevent mold growth without permanently inactivating the embryo and there was some evidence that the concentration of the inhibitors necessary to prevent incubation and growth of fungi was low enough to allow respiration of wheat.

Experimental

In this study the rate of respiration was estimated by determining the carbon dioxide production of wheat at various moisture contents isothermally and by observing the temperature changes in 15-lb. samples of damp wheat stored in insulated containers.

In the measurement of carbon dioxide production, two general methods were used; in one the samples of wheat were allowed to remain in closed containers for 23 hr. and the carbon dioxide was aspirated off for one hour; in the other carbon dioxide free air was drawn through the wheat continuously except for the time required to make a titration each 24 hr. With both these procedures the carbon dioxide was absorbed in standard barium hydroxide solution.

In the measurement of temperature changes it was necessary to use quite large quantities of wheat. After a number of preliminary trials it was found that 15-lb. samples in earthenware crocks, heavily insulated with wood-shavings, would show temperature rises at certain moisture contents. The temperatures were measured by means of long-stemmed accurate mercury thermometers inserted in the centre of the mass of wheat.

Comparison of the Continuous and Discontinuous Methods for Removal of Carbon Dioxide

In making a choice between the continuous and discontinuous methods for studying the carbon dioxide production of damp wheat, a number of facts must be considered. In the first place, a discontinuous method more closely approximates actual commercial conditions, in which the grain is stored in bins with no great chance of aeration. Furthermore this method permits the handling of a large number of samples with only one or two absorption trains. On the other hand, it has been generally recognized that accumulation of carbon dioxide in damp wheat tends to retard the respiration process and therefore the discontinuous method does not give the maximum rate of carbon dioxide production for a given moisture content and temperature. Commercially stored wheat may be subjected to "turning", both in the process of handling and for the express purpose of cooling the wheat, and thus it undergoes varying degrees of aeration. In order to estimate the difference between the two methods, determinations were made for three days on wheat at 20% moisture content. The results, given in Table I, show a very great difference in respiration rate. The continuously aerated sample not only was higher on the first day, but also increased in rate more rapidly than the non-aerated sample.

TABLE I

COMPARISON OF THE CONTINUOUS AND DISCONTINUOUS AERATION METHODS OF CARBON DIOXIDE DETERMINATION

(Wheat at 20% moisture content)

Days after tempering	Mg. of CO_2 per 100 gm. of dry wheat per 24 hr.	
	Continuous aeration	Discontinuous aeration (CO_2 swept out at end of 23 hr.)
7	59.6	21.0
8	81.4	19.6
9	131.8	39.3

TABLE II

EFFECT OF SIZE OF THE CONTAINER ON RESPIRATION RATE OF A GIVEN QUANTITY OF WHEAT

(500 gm. wheat at 20% moisture content in each case. This quantity of wheat occupied approximately 600 cc.)

Days after tempering	Mg. CO_2 per 24 hr. per 100 gm. dry wheat		
	650-cc. bottle	1000-cc. bottle	2000-cc. bottle
1	18	18	18
2	28	24	23
3	37	39	36
4	38	63	53
5	40	67	77
6	39	64	95
7	37	61	103
	Half of each sample of wheat was removed		
8	62	90	141
9	74	100	144
10	85	99	137
11	92	101	141

Furthermore, it was observed that with the discontinuous method the rate of carbon dioxide production depended to some extent on the free air space in the vessel. This is illustrated by the data in Table II, which show, as would be expected, that increasing the size of the container for a given quantity of wheat increases the amount of carbon dioxide produced. This indicates that factors such as weight per bushel and degree of packing the sample would affect the rate of carbon dioxide production in small containers.

For comparative work the continuous method seemed less subject to error and accordingly was adopted.

Acceleration of the Rate of Carbon Dioxide Production

Before proceeding to a discussion of the effect of moisture on the respiration rate, it is necessary to consider the time effect. Bailey and Gurjar (1) allowed the tempered wheat to stand for three days and then sealed the samples in containers and left them for four days, after which the carbon dioxide was aspirated off and estimated. In most of their work the rates of carbon dioxide production were calculated on the basis of this four-day period. The data in Table II show that after about three days from tempering the rate of carbon dioxide production in the two smaller containers approached a constant value which varied with the free air space in the container. In the largest bottle, which had about 1400 cc. free space above the wheat, there was an increase in rate of carbon dioxide production in each successive 24-hr. period for the first seven days. This is similar to the observations made by the continuous aeration method. It is evident, therefore, that given the proper conditions, damp wheat tends to increase in rate of carbon dioxide production; it certainly does not give a constant rate four days after tempering, except when the respiration is inhibited by the presence of carbon dioxide in too great concentration. The acceleration of rate of carbon dioxide production was not due to heating of the wheat, because the samples were too small; many measurements showed that under the conditions of these experiments the wheat did not get above room temperature, which was maintained at 22 ± 1° C. This effect, therefore, must have been due either to progressive stimulation of embryonic activity or to fungal growth. Examina-

tion disclosed the fact that samples which had "respired" at a high rate were heavily infected with fungi, mostly of the *Penicillium* type, with a few *Aspergilli*. Means were then sought for destroying the fungus spores or of inhibiting their germination and growth.

The Effect of Toluene on Carbon Dioxide Production

The work of Tomkins (18) has thrown considerable light on the action of gases and volatile substances on the growth of mold fungi. Working with acetone, acetaldehyde, hydrogen cyanide, hydrogen sulphide, sulphur dioxide and ammonia, he found that these substances either retarded the germination of the spores or inhibited their growth, or both. Swanson (17) showed that Ceresan, the active principle of which is ethyl mercuric chloride, is effective in preventing mold growth. As none of these reagents seemed to be suitable for use on milling wheat, especially on a commercial scale, it was decided to try the effect of other, more suitable substances, and the first chosen for investigation was toluene.

By the continuous aeration method, toluene vapor was introduced into the air stream by passing the air through a bubbler containing liquid toluene. The concentration of vapor thus obtained was not high; a 24-hr. run vaporized approximately 1 cc. of the liquid. In all tests of fungus inhibitors a control consisting of untreated wheat of the same lot was tested at the same time. A typical set of data obtained on toluene-treated and control samples of wheat tempered to 20% moisture content is given in Table III. In these data it should be noted that with both the treated and untreated samples, the average rate of carbon dioxide production for the fourth, fifth, sixth and seventh days after tempering was practically the same. This corresponds to the period during which Bailey and Gurjar (1) made their measurements of respiration rate. It seems likely that at 22° C., growth of the fungi does not get under way rapidly until after seven days from tempering, in wheat of 20% moisture content. If this conclusion is correct, it can be stated further that a four-day exposure to toluene vapor has no deleterious effect on the true respiration of the wheat itself, because during this period the treated and untreated samples showed the same carbon dioxide production rate. After the

TABLE III

EFFECT OF TOLUENE VAPOR ON CARBON DIOXIDE PRODUCTION OF WHEAT AT 20% MOISTURE

Days after tempering	Mg. CO_2 per 100 gm. dry wheat per 24 hr.			
	With toluene		Control—without toluene	
2	23.6		—	
3	25.4		30.4	
4	27.9	Average 29.1	27.3	Average 28.1
5	27.7		28.9	
6	28.7		26.2	
7	32.3		36.0	
8	32.8		63.8	
9	36.8		105.0	
10	32.0		133.0	
11	33.2		160.8*	
12	39.8		77.8	
13	36.5		43.9	
14	30.8		37.1	

**Toluene added after this determination was completed.*

seventh day, however, the rate of carbon dioxide production in the control sample increased very rapidly until on the eleventh day it was about 5.3 times the initial rate. During the same period, the amount produced by the toluene-treated sample increased from 32.3 to 33.2 mg. per 100 gm. per 24 hr., or from the average rate of 29 to 33, an increase that is likely within the experimental error of the method. It was concluded that the toluene vapor had either killed the spores themselves or the fungi as fast as they germinated or had simply inhibited the germination of the spores. Introduction of toluene vapor to the control sample at the end of the eleventh day resulted in a rapid diminution of carbon dioxide production and by the fourteenth day the rate on the control had dropped from 161 to 37. This was considered as evidence that toluene vapor either killed the fungi or reduced their respiratory activity to negligible proportions. It will be seen later that the second alternative is the more probable.

An attempt was made to surface sterilize the wheat, in order to see if it would be possible to get an estimate of the true respiration rate, without having to use toluene. Mead (8) in a study of various sterilizing agents found that silver nitrate and mercuric chloride solutions effected very good sterilization in respect to fungi, without seriously damaging the germination. Accordingly a sample of wheat was immersed in 0.1% mercuric chloride solution for 10 min., after which the grain was thoroughly washed. Carbon dioxide production of the samples with and without toluene was measured. The results are given in Table IV.

TABLE IV

CARBON DIOXIDE PRODUCTION OF WHEAT OF 25% MOISTURE CONTENT, PREVIOUSLY IMMERSED FOR 10 MIN. IN 0.1% MERCURIC CHLORIDE SOLUTION AND THEN WASHED

Days after tempering	Mg. CO_2 per 100 gm. dry wheat per 24 hr.	
	With toluene	Control—without toluene
4	120	82
5	125	86
6	122	86
7	97	77
8	76	71
9	58	70
10	41	71
11	28	74
12	24	87
13	25	119
14	16	133
15	11	167
16	9	181
17	7	222

Examination of the samples after the run had been made showed the following:

Fungus infection before incubation	0%	31%
Infection after incubation	93% (mostly bacteria)	98% (mostly *Penicillium*)
Germination	0%	0.5%

The samples treated with toluene vapor commenced producing carbon dioxide at a higher rate than the control samples, but as time went on the rate decreased, until finally on the seventeenth day after tempering, the rate was down to 7. The control samples showed a tendency to decrease in rate

until the thirteenth day, when the trend reversed and a rapid increase occurred during the last five days of the experiment. The initial stimulation which was apparently due to the toluene vapor is analogous to results obtained by Passerini (12), who observed that a short immersion of seeds in carbon disulphide or carbon tetrachloride accelerated germination. While slight increases in carbon dioxide production, attributable to toluene or carbon tetrachloride vapor, have been observed in many instances, at no time has the effect been as great as in this particular case. It can only be assumed that the immersion in the mercuric chloride solution made the embryo more sensitive to stimulation.

After the respiration "run" was finished, the samples of wheat were submitted to Mr. Mead for examination; his observations are given at the bottom of Table IV. The wheat had entirely lost its viability; the sample treated with toluene vapor showed no fungus infection, but after incubation 93% of the kernels were found to be infected with bacteria; the control sample was 31% infected with fungal growth and after incubation 98% of the kernels examined were infected, mostly with *Penicillium*. It is obvious from these data that the treatment with mercuric chloride solution was ineffective as a sterilizer for fungi. Furthermore, it weakened the viability of the embryo to such an extent that exposure to toluene vapor on the one hand, and growth of fungi on the other, destroyed the germination entirely. It would therefore be out of the question to attempt to measure the true respiration rate by this method.

In order to ascertain the effect of exposure to toluene vapor on the viability of wheat the following experiment was made. Samples of the one lot of wheat were tempered to moisture contents of 10.5, 12.5, 15, 17.5, 20.0, 22.5 and 25%. These were placed in sealers at the bottoms of which were open beakers protected by wire gauze, containing toluene, and left for four days, after which time they were exposed and spread out in the laboratory for two days. Germination tests made on these samples gave the results shown in Table V.

It is evident that exposure to toluene vapor tends to inactivate the embryo permanently and, therefore, while it is effective in preventing growth of molds, it could not be applied in this study because of the difficulty of estimating how much of the retardation of carbon dioxide production was due to inhibition of molds and how much to inactivation of the embryo. The fact that it lowers the viability seriously and furthermore that it is inflammable renders toluene inapplicable commercially.

TABLE V

GERMINATION TESTS ON WHEAT OF VARIOUS MOISTURE CONTENTS PREVIOUSLY EXPOSED TO TOLUENE VAPOR FOR FOUR DAYS

Moisture, %	Germination, %
25	16
22.5	15
20	35
17.5	33
15	60
12.5	70
10.5	64
Control sample	92

In some tests made with a non-inflammable mixture of toluene and carbon tetrachloride it was found that the mixture prevented mold growth as effectively as the pure toluene. This led further to the observation that pure carbon tetrachloride was an efficient fungus growth inhibitor. Thereafter attention was directed solely to the investigation of the behavior of carbon tetrachloride on damp wheat, because this substance, if effective, possesses characteristics making it ideally suited to commercial application; it is low in price, is non-inflammable, being in fact used extensively as a fire extinguisher, and is reputed to be a fairly good insect repellent. If it could be applied in effective concentration without damaging the wheat for storage or for milling and baking purposes, it might be used commercially to prevent damage to damp wheat in transit to the terminal elevators.

The Effect of Carbon Tetrachloride on Carbon Dioxide Production of Damp Wheat

Carbon dioxide production measurements were made on samples of wheat tempered to 12, 14, 16, 18, 20, 22 and 24% respectively. The runs were started in each case three days after tempering. The carbon tetrachloride vapor was introduced by means of a bubbler containing water and carbon tetrachloride. Runs were continued for eight days, after which the wheat was examined carefully for signs of mold and was given a germination test. The observations are presented in Table VI.

The carbon tetrachloride vapor prevented mold growth in all except the 24% moisture sample. No molding of the control samples occurred at 16% or lower moisture, but at 18% and higher moistures the wheat molded and the germination was impaired. The samples exposed to carbon tetrachloride vapor showed no evidence of reduction of viability except in the case of the 24% sample. This sample, which was at a higher moisture than is ordinarily found in commercial samples of damp wheat, probably should not have been allowed to stand for three days after tempering, because at this high moisture content germination of spores might have started.

Attention should be directed to the progressive decrease in carbon dioxide production rate of the higher moisture samples treated with carbon tetrachloride vapor. At first this looked like a decrease in embryonic activity resulting from prolonged exposure to the vapor, but the germination data belie the suggestion of damage to the embryo. Another explanation of this decrease in rate might be sought in the decrease in moisture content of the samples during the course of the experiment. This change in moisture content is a serious difficulty with the continuous aeration method and can be overcome only by using solutions the vapor pressure of which is in equilibrium with the wheat sample under examination. At the time these experiments were being conducted there was not sufficient information available on this subject, and consequently the data herein presented must be discounted somewhat. However, the drop in moisture content is not adequate as an explanation of the decrease in rate of carbon dioxide production noted

TABLE VI

EFFECT OF CARBON TETRACHLORIDE VAPOR ON THE RATE OF CARBON DIOXIDE PRODUCTION AND ON GERMINATION OF WHEAT AT VARIOUS MOISTURE CONTENTS

Initial moisture content	Mg. CO_2 per 24 hr. per 100 gm. wheat													
	12%		14%		16%		18%		20%		22%		24%	
Days after tempering	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4
4	4.5	3.2	4.5	2.6	6.7	4.4	17.5	5.5	18.4	19.4	48	37	74	100
5	3.9	2.6	4.0	2.6	4.0	3.4	21.3	6.2	18.4	16.3	51	34	87	111
6	3.9	2.6	3.3	2.6	4.7	3.4	33.2	5.5	19.8	14.1	48	31	95	98
7	3.2	3.2	5.2	2.6	5.1	3.4	41.4	4.8	28.3	12.4	47	29	104	87
8	2.6	2.0	3.3	2.6	5.1	3.4	43.5	5.5	37.4	10.6	52	28	113	80
9	4.5	3.2	4.0	3.3	6.7	4.0	44.2	4.2	42.3	7.8	64	27	120	73
10					5.4	4.0	44.2	3.5	48.1	8.4	81	26	136	75
11					4.0	3.4	47.0	3.5	50.9	8.1	96	28	149	75
Moisture at end of run	12.3	12.0	13.8	13.3	15.6	15.7	19.5	17.5	19.9	19.1	22.3	21.4	23.8	23.9
Germination after the run	92	100			96	92	76	92	68	98	32	92	32	62
Condition after the run	normal	normal	normal	normal	normal	normal	moldy odor	normal	quite moldy	normal	very moldy	normal	very moldy	trace moldy

in Table VI, because in the case of the 24% moisture sample, in which the decrease of rate was greatest, there was an insignificant decrease in moisture content of the wheat.

There are two other possible explanations, one being that the first effect of carbon tetrachloride vapor is a stimulation of the embryo, the other being that there is a progressive inhibition of respiratory activity, an anaesthesia effect, which slows down the metabolism without injuring the organism. This effect is noticeable only in the samples of higher moisture content. The anaesthesia explanation gets some support from the fact that wheat will not germinate in an atmosphere even of relatively low carbon tetrachloride vapor concentration, but on change from that atmosphere to one free from the vapor will germinate 67%. Results of such an experiment are given in Table VII.

TABLE VII

EFFECT OF CARBON TETRACHLORIDE VAPOR ON GERMINATION OF WHEAT

Treatment	Germination, %
1. Control	100
2. 0.1 cc. CCl_4 in 10 litre vessel	92
3. 1.0 cc. CCl_4 in 10 litre vessel	0
4. Sample 3 in 10 litre vessel free from CCl_4 vapor	67

In each case the germination tests were made in a 10 litre desiccator with closely fitting top. For No. 4, the sample was removed and blown free of carbon tetrachloride vapor and the vessel was carefully swept free of the vapor; the sample was then returned and tested in the usual way. The lowered viability may have been due to actual damage to the embryo or to residual carbon tetrachloride that had become absorbed by the wet wheat. It will be shown later that damp wheat does absorb the vapor to considerable extent. The important fact in these data is that, although the wheat was still viable, it gave not the least sign of germination in an atmosphere in which the concentration of carbon tetrachloride vapor could not have been greater than 18 cc. per 1000 cc., provided all the carbon tetrachloride evaporated at once and none of the vapor was removed by absorption or hydrolysis. It seems quite probable, therefore, that the decreases noted in Table VI may be accounted for, in part at least, by assuming that carbon tetrachloride vapor tends to inhibit embryonic activity.

The foregoing discussion points to the conclusion that a relatively low concentration of carbon tetrachloride vapor constantly maintained is quite effective in preventing the usual rapid increase in carbon dioxide production associated with germination and growth of fungi in wheat of 18% or higher moisture content, and that such effective concentrations of carbon tetrachloride vapor have little or no deleterious effect on the viability of the wheat. In considering the moisture levels in Table VI, it should be kept in mind that these determinations were conducted under isothermal conditions, not at all comparable to those to be found in large bulks of grain. Therefore, no conclusions can be drawn from these data as to the safe moisture limits of

untreated commercial damp wheat. It can be said, however, that wheat of 18% moisture content can be stored without danger of damage by heating if treated with an adequate concentration of carbon tetrachloride vapor, because under these conditions the carbon dioxide production rate is of the same order as that of 12% wheat. It will be shown later, in the discussion of "heating of damp wheat", that wheat at 25% moisture content can be prevented from heating by proper use of carbon tetrachloride, and on this basis, it may be stated tentatively that the carbon dioxide production rates shown in Table VI for samples at 22 and 24% moisture treated with carbon tetrachloride are likely too low to start heating, but there is no direct evidence for this assumption.

The Effect of Moisture on Carbon Dioxide Production of Wheat

The term "carbon dioxide production" has been used in place of "respiration" because it is evident that there are two kinds of respiration in damp wheat, one due to the slow metabolism of the semi-dormant embryo of the wheat, the other due to the active metabolism of the rapidly growing fungi. From the standpoint of the commercial problem of heating it is important to separate these two respirations, especially if it is found possible to control the activity of fungi, because it would be necessary to ascertain whether the respiration of the wheat alone could produce enough heat to affect the condition of the grain seriously. If we consider the problem as it affects the present methods of handling damp wheat, it probably is not necessary to differentiate these respirations, because they both work to the same end, namely, an increase in temperature of the mass of wheat, accompanied by acceleration of respiration of both the wheat and the fungi until a maximum temperature is reached, by which time the grain has become definitely spoiled. Whichever way the problem is regarded, it will be very difficult to come to a definite conclusion.

From the data in Table VI, it is evident that there is no tendency toward acceleration of rate in either the treated or control samples at or below a moisture content of 16%. At some moisture value between 16 and 20% the control samples showed a marked acceleration of rate. Unfortunately the control at 18% underwent an unaccountable increase in moisture content during the course of the run, getting up to 19.5% at the finish, and the values for that particular sample are unreliable. However, judging from the rates shown by the treated sample, it would seem reasonable to suppose that the rapid acceleration starts at moistures closer to 20% than to 16%. It is not important to establish this point definitely for laboratory conditions because, as mentioned before, these observations were made on small samples of grain under approximately isothermal conditions. Furthermore, the moisture point at which a rapid acceleration starts must vary with other factors such as temperature, kind, and soundness of grain.

It is important to know that beyond some definite critical moisture limit the rate of total carbon dioxide production increases very sharply and acceler-

ates with time even under isothermal conditions. This acceleration is doubtless due to multiplication of fungi. The differences between the carbon dioxide production rate on the fourth and eleventh days give some measure of this effect, but the difference cannot be attributed wholly to the respiration of the fungi, because we cannot say definitely that the rate on the fourth day after tempering represents only wheat respiration. It may be stated emphatically that the carbon dioxide measurements on wheat of 22% moisture or lower, taken on the fourth and fifth days after tempering, give as good an estimate of wheat respiration at 22° C. as can be obtained.

The rates given by the treated samples are probably lower than the true respiration rate for wheat at these moistures, because, as pointed out previously, there is evidence for believing that carbon tetrachloride vapor tends to inhibit embryo activity in the wheat, especially at the higher moistures. The differences between the carbon dioxide production rates of the treated and check samples on the eleventh day represent the controllable part of the total carbon dioxide production of damp wheat. It should be particularly noted that the action of carbon tetrachloride vapor raises the critical moisture limit by at least 2%, in this case definitely from 16% to 18 or probably 20%. This means that wheat at 18% moisture content respires no faster in the presence of carbon tetrachloride vapor than wheat at 12% moisture and therefore *could not heat* under these conditions. Even at 20% the initial high rate of 19 is reduced in nine days to a rate of 8 which would seem to be low enough to preclude the possibility of heating. Indeed it seems extremely probable that the highest final rate recorded for the treated samples, namely 75, is not sufficient to cause heating if fungal growth can be prevented. Evidence for this is to be found in the discussion of heating which follows.

The Heating of Damp Wheat

Since reference to the methods used to study heating of wheat has been made in the first part of the paper, consideration of the results most pertinent to this subject may now be undertaken without discussion of the many preliminary experiments.

The Effect of Moisture on Heating

In order to get some information regarding the time required to reach maximum temperature, four 15-lb. samples were made up to 16.1, 18.1, 19.5 and 21.5% moisture content respectively and kept under observation for 19 days. The data obtained are given in Table VIII.

At 16.1% moisture the temperature of the wheat did not rise more than 2° C. above room temperature; at 18.1% moisture the temperature started rising slowly at the seventh day and by the nineteenth day had risen to about 31° C., eight degrees above room temperature; at 19.5% moisture a maximum of 44.7° C. was recorded on the sixteenth day; at 21.5% moisture a maximum of 44.5° C. was recorded on the ninth day.

TABLE VIII

TEMPERATURE OF WHEAT STORED AT VARIOUS MOISTURE CONTENTS

Days	Temperature of sample in °C. 1	2	3	4
0	23.7	23.2	24.0	23.8
2	20.0	20.5	21.3	22.0
4	20.2	21.1	21.4	26.0
5	20.5	22.0	22.7	29.0
7	21.2	23.0	28.2	36.0
9	22.0	24.5	35.5	44.5
10	22.2	26.0	39.0	44.0
11	22.5	26.0	39.0	43.0
12	22.5	27.0	40.0	43.0
16	24.0	31.0	44.7	44.0
17	24.2	30.5	44.0	42.7
18	24.4	30.7	43.3	41.6
19	24.2	30.3	42.6	41.3
Moisture content	16.1%	18.1%	19.5%	21.5%
Final condition	sound in appearance, slight yeasty odor	pronounced musty odor	both severely bin-burned and completely spoiled	

While a difference of seven days in time was required to reach the maximum temperature of approximately 45° C. with the samples of 19.5% and 21.5% moisture content, there was only a difference of 2-3 days in time required for initiation of heating. The rate of heating, therefore, appears to be directly related to the moisture content of the wheat. It should be pointed out, too, that there was a distinct maximum of about 45° C.; after this was attained there was a tendency to decrease in temperature. This may be attributable to destruction of the embryo activity of the wheat or to killing of the fungi, or to both. It agrees with the data of Smith and Bartz (15), which show that cracked corn and crushed oats, when stored in piled sacks, attained maximum temperatures varying from 42° to 49° C., after which there was a slow decrease.

The condition of the samples at the end of the experiment is briefly described in Table VIII. Those at 19.5 and 21.5% moisture were typically bin-burned and were considered completely ruined, even for feed. The sample at 18.1% which had reached a maximum of only 31° C. had a musty odor sufficiently pronounced to degrade it to "Feed". These three samples were heavily infected with spores. In the light of the "respiration" results it appeared probable that fungus growth was responsible to a large extent for the increased carbon dioxide production which led to the increased temperatures, but since ordinary wheat respiration might produce the same temperature effect under the semi-adiabatic conditions of these experiments no definite conclusion on this point could be reached from consideration of these particular data. It was definitely shown in another experiment that initial inoculation of a 15-lb sample with 100 gm. of previously heated wheat had the effect of greatly hastening the attainment of the maximum tem-

perature. Furthermore, as will be shown by the results of the following experiment, damp wheat can be treated with carbon tetrachloride so as to prevent heating and spoilage. These considerations lead to the conclusion that growth of mold is mainly responsible for the heating of damp wheat.

Prevention of Heating of Damp Wheat

In order to reduce time and to compensate to some extent for the small bulk of the samples, the wheat used in the following experiment was tempered to 25% moisture content, a value seldom reached in commercial lots of damp wheat. The carbon tetrachloride was applied by means of a small flask containing 75 cc. of the liquid, into which dipped a loose wick of cotton wool. The top of the flask was guarded by a copper gauze hood. This crude evaporator was placed in the bottom of the 3-gallon crock and covered with 15 lb. of the wheat. Observations were made for 13 days and then the samples were unpacked and examined. All the carbon tetrachloride had evaporated and these treated samples showed very faint signs of mold. The evaporators were replenished, the wheat put back and observations continued for 10 days more. The temperatures recorded are given in Table IX.

TABLE IX

EFFECT OF VARIOUS TREATMENTS ON THE TEMPERATURE OF DAMP WHEAT; 15-LB. SAMPLES OF WHEAT AT 25% MOISTURE CONTENT WERE USED

Days after tempering	1 Control	2 CCl_4 vapor present	3 CCl_4 vapor present	4 In sealed container	Room temperature
1	25.6	25.6	25.2	27.5	21.8
2	24.9	23.3	23.5	26.3	21.7
3	24.8	22.8	23.0	26.0	21.8
4	25.6	22.6	22.8	26.1	22.0
5	27.9	22.4	22.8	26.1	22.0
6	32.7	22.3	22.8	26.0	21.6
7	39.5	22.1	23.0	25.3	21.0
8	43.9	21.8	21.8	25.0	20.6
9	45.8	21.6	21.5	25.0	21.0
10	45.9	21.6	21.5	25.2	21.5
11	45.2	21.9	22.0	25.5	22.2
12	44.2	22.1	22.3	25.5	21.7
13	—	22.0	22.2	—	21.5
17	—	22.7	22.5	25.1	
19	—	22.4	22.5	24.3	
21	—	21.6	21.5	23.8	
23	—	21.1	21.1		
Final condition	completely spoiled	normal	normal	musty	

The control sample reached a maximum temperature of approximately 46° C. in nine days, but the samples exposed to the carbon tetrachloride vapor showed absolutely no tendency to increase in temperature. They were about 3.5° C. above room temperature on the first day, on account of the heat of wetting, but this initial temperature had decreased to room tem-

perature by the fifth day and thereafter there was no significant increase. The final examination of these treated samples showed them to be normal in appearance, with no musty odor.

Along with these samples, there was carried one untreated sample sealed in an airtight container filled to its capacity with 20 lb. of wheat at 25% moisture. This sample showed no tendency to heat, but rather a slight tendency to drop in temperature. During the greater part of the 23-day period it was about 4° C. above room temperature. On final examination the sample was found to have a musty odor. It is evident that in sealed containers the carbon dioxide production is sufficiently retarded to prevent any marked rise in temperature, but under these conditions enough mold growth may occur to put the grain out of condition.

While the foregoing experiment furnished convincing evidence that heating of damp wheat can be prevented by carbon tetrachloride vapor, it was recognized that the production and maintenance of an effective concentration of the vapor in lots of wheat of commercial size would present a number of difficulties. Initial spraying of the wheat with carbon tetrachloride by means of an atomizer was found to be ineffective; it seems necessary to maintain continuously a certain concentration of the vapor. This might be expected on account of the fact that carbon tetrachloride does not kill the spores, but only inhibits their development. It is necessary, therefore, to use some sort of evaporator which can be depended on to supply the vapor constantly. The question then arises, how far will the vapor penetrate in damp wheat; in other words, what would be the effective range of an evaporator? On account of the great relative density of carbon tetrachloride vapor one would expect that downward displacement might be a little more rapid than upward displacement. However, Strand (16) showed that adsorption of the vapor by the top layers of grain prevents its rapid downward movement and consequently, when the vapor is applied at the top of a column of grain, the concentration of the gas varies inversely with the depth below the surface. In the aforementioned heating trials, the top of the evaporator was not more than 10 in. below the surface of the grain and the container was loosely covered with shavings to lessen air movement. Under these conditions, there evidently was maintained a concentration of carbon tetrachloride vapor sufficient to prevent mold growth, which indicated that the vapor was not adsorbed too rapidly for practical purposes. In order to get more information concerning the effective penetration of carbon tetrachloride vapor in damp wheat, the following trials were made.

Cylindrical towers were constructed of heavy waxed paper cartons 8 in. high and 6 in. in diameter. The bottom of each carton was cut away and replaced by copper gauze and they were joined by means of cardboard bands in such a way that the whole tower could be put up and taken down in segments. In setting up a run each segment was filled to capacity, so that the bottom of the upper one rested on the top of the wheat in the one below. This was as close an approach to a continuous column of wheat as could be

arranged with the equipment at hand. Of course, after charging, the wheat in each segment tended to settle, with the result that finally there were always small air spaces between the segments. Doubtless, too, there was opportunity for leakages of gas around the joints. However, it was thought that loss of vapor might compensate to some extent for lack of continuity of the mass, and at any rate the results would give some indication of the extent of effective penetration of the carbon tetrachloride vapor. Experiments were conducted with the source of carbon tetrachloride both at the bottom and at the top of the tower. Some of the results are presented in Tables X and XI.

TABLE X

UPWARD PENETRATION OF CARBON TETRACHLORIDE VAPOR IN DAMP WHEAT. OBSERVATIONS MADE ON WHEAT AT 19% INITIAL MOISTURE, IN VERTICAL SEGMENTED TOWERS, 6 IN. IN DIAMETER, EACH SEGMENT 8 IN. HIGH. SEGMENTS NUMBERED CONSECUTIVELY FROM THE BOTTOM UP. 200 CC. CARBON TETRACHLORIDE IN WIDE BEAKER PLACED AT BOTTOM

(Duration of experiment—10 days)

Segment	Appearance of wheat	Odor of CCl_4	Odor of mold	Final moisture content
1	sound	marked	nil	17.5
2	sound	detectable	nil	17.8
3	sound	doubtful	faint	17.8
4	some mycelia	nil	marked	17.4
5	some mycelia	nil	marked	17.4
6–13	moldy	nil	marked	17.4–15.5

During the ten days 103 cc. of CCl_4 evaporated. The fungal growth was most pronounced at the top of each segment.

TABLE XI

DOWNWARD PENETRATION OF CARBON TETRACHLORIDE VAPOR IN DAMP WHEAT. EXPERIMENT CONDUCTED AS DESCRIBED IN TABLE X EXCEPT THAT 100 CC. CARBON TETRACHLORIDE WAS POURED INTO THE TOP OF THE COLUMN AND A BEAKER WITH 225 CC. CCl_4 WAS PLACED IN THE TOP SEGMENT.

WHEAT AT 20% MOISTURE CONTENT

(Duration of experiment—7 days)

Segment	Appearance of wheat	Odor of CCl_4	Odor of mold
12—blank			
11	sound	strong	nil
10	sound	strong	nil
9	sound	strong	nil
8	sound	strong	nil
7	sound	fair	very slight
6	sound	slight	very slight
5	sound	slight	very slight
4	sound	doubtful	slightly stronger than 5
3	sound	slight	nil
2	sound	slight	very slight
1—blank			

By the second day the odor of CCl_4 was very distinct at the bottom and throughout the column. The CCl_4 evaporated at the rate of 20 cc. per day. There were no visible signs of mold, and on drying this wheat appeared sound.

The two sets of observations cannot be compared, because in one case, the downward diffusion experiment, the wheat was initially treated by pouring 100 cc. of liquid carbon tetrachloride into the top of the tower. It is evident, however, from Table X that the effective upward penetration of carbon tetrachloride vapor under these conditions was not more than 24 in. On the other hand, by the method described in

Table XI it was possible to maintain a fairly effective concentration of the vapor through a distance of 72 in. of wheat.

This experiment is scarcely comparable to commercial conditions, because the bottom segment was above the floor and had a small door. This might tend to facilitate the downward movement of the vapor. The trial does show, however, that enough carbon tetrachloride evaporates from a small surface to create and maintain a concentration of vapor effective in preventing mold growth even in wheat of 25% moisture content.

As a result of these and other similar observations, it is concluded that the application of carbon tetrachloride can be made most effectively by first adding the liquid, either by pouring or spraying it over the wheat *in situ* or as it is being loaded, and then maintaining a source of carbon tetrachloride at the top of the mass of wheat. It is recognized that these investigations are at best only approximations to commercial conditions and that extensive tests on large bulks of damp wheat must be conducted before a definite recommendation regarding a practical method can be made.

The Effect of Carbon Tetrachloride on the Milling and Baking Quality of Wheat

In considering the possibility of applying this treatment commercially it is essential to know how such treatment might affect the quality of flour. Accordingly a careful study was made of flour milled from wheat treated with varying degrees of severity with carbon tetrachloride.

Five 5-lb. samples of sound wheat were tempered to 24% moisture content, placed in securely stoppered bottles, to which were added dosages of 1, 2, 4, 8 and 12 cc. carbon tetrachloride respectively. They were left for 25 days and then dried and milled in the usual way. With this series there were included three control samples, one of the untreated original wheat at 12% moisture, one of the same wheat treated for 25 days with 4 cc. of carbon tetrachloride and one of untreated wheat of 24% moisture content. The miller's observations are given in Table XII.

TABLE XII

OBSERVATIONS ON THE ODOR OF FLOUR AND TASTE OF BREAD PREPARED FROM WHEAT TREATED WITH CARBON TETRACHLORIDE

Description of treatment of sample	Odor of flour	Taste of bread
1. Control (1)—original wheat at 12% moisture	normal	normal
2. Control (2)—original wheat at 12% moisture with 4 cc. CCl_4	normal	normal
3. Control (3)—24% moisture	slightly musty	normal
4. 24% moisture with 1 cc. of CCl_4	fruity odor	normal
5. 24% moisture with 2 cc. of CCl_4	fruity odor	normal
6. 24% moisture with 4 cc. of CCl_4	fruity odor	normal
7. 24% moisture with 8 cc. of CCl_4	odor of CCl_4	normal
8. 24% moisture with 12 cc. of CCl_4	odor of CCl_4	normal

After being aged for three weeks, the flours were baked by six different formulas which are described in Table XIII.

TABLE XIII

EFFECT OF TREATMENT WITH CARBON TETRACHLORIDE ON BAKING QUALITY

Description of sample	Absorption, %	Loaf volume, cc.	Texture	Crumb color	Crust color	Shape
	Simple formula					
1. Control (1)—original wheat at 12% moisture	62	725	7.5	7.5	5	5
2. Control (2)—original wheat at 12% moisture with 4 cc. CCl_4	62	650	7.5	7.5	3	4
3. Control (3)—24% moisture	62	695	6.5	7	4	4
4. 24% moisture with 1 cc. CCl_4	61	760	7.5	7.5	4	5
5. 24% moisture with 2 cc. CCl_4	60	735	7.5	8	5	5
6. 24% moisture with 4 cc. CCl_4	60	730	7	8	5	5
7. 24% moisture with 8 cc. CCl_4	61	715	7.5	7.5	4-d**	5
8. 24% moisture with 12 cc. CCl_4	61	743	7	8	4-d	5
	0.001% $KBrO_3$					
1. Control (1)—as above	62	768	7.5-o*	8	4-d	5
2. Control (2)—as above	62	808	7-o	8	4-d	5
3. Control (3)—as above	62	835	7-o	7.5	4-d	5
4. As above	61	900	6.5-o	8	4-d	5
5. As above	60	838	7-o	8	4-d	5
6. As above	60	830	7-o	8	4-d	5
7. As above	61	798	7-o	8	4-d	5
8. As above	61	866	7-o	9	4-d	5
	0.002% $KBrO_3$					
1. Control (1)—as above	62	875	5-o	8	4-d	5
2. Control (2)—as above	62	878	5-o	8	4-d	5
3. Control (3)—as above	62	773	5-o	8.5	4-d	5
4. As above	61	783	7-o	8.5	4	5
5. As above	60	783	7.5-o	8.5	5	5
6. As above	60	783	7.5-o	8.5	4-d	5
7. As above	61	770	7-o	9	4-d	5
8. As above	61	750	6.5-o	8.5	5	5
	0.003% $KBrO_3$					
1. Control (1)—as above	62	705	7.5	8	5	4
2. Control (2)—as above	62	780	8-o	9	5	4.5
3. Control (3)—as above	62	760	7	8	5	5
4. As above	61	690	7.5	8	5	4.5
5. As above	60	715	7.5	8	5	5
6. As above	60	695	7.5	9	5	4.5
7. As above	61	685	7.5	9	5	5 torn
8. As above	61	670	7.5	9	5	3.5 torn
	0.004% $KBrO_3$					
1. Control (1)—as above	62	770	7	7	5	3 torn
2. Control (2)—as above	62	750	8-o	8	4	5
3. Control (3)—as above	62	630	7	7	4	3
4. As above	61	633	8-o	8	4	3
5. As above	60	619	7	8	4	3
6. As above	60	645	7.5	8	4	4
7. As above	61	618	8	8	4	3
8. As above	61	590	8.5	7	4	3
	Malt-bromate-phosphate					
1. Control (1)—as above	62	1090	5	8	4-d	4
2. Control (2)—as above	62	1010	5-o	9	3-d	4
3. Control (3)—as above	62	830	7	8	5	5
4. As above	61	805	6.5	8	5	4
5. As above	60	865	7-o	8	5	4
6. As above	60	800	7	9	5	5
7. As above	61	750	7	8	4-d	5
8. As above	61	920	7	8	4-d	4

*o = open. **d = dark.

With the basic formula no deleterious effect of the carbon tetrachloride treatment was observed; on the contrary, there was a very slight evidence of improvement.

Four straight bromate formulas with 1, 2, 3 and 4 mg. potassium bromate respectively were used. With the lower dosage, the effect of carbon tetrachloride treatment was, if anything, favorable. With increasing dosages of potassium bromate, however, the samples that had been tempered to 24% moisture content commenced falling below the 12% moisture samples. However, comparison with the 24% moisture control sample failed to show any specific effect attributable to the carbon tetrachloride treatment.

With the malt-bromate-phosphate formula, again, all those samples initially tempered to 24% gave volumes about 200 cc. lower than the dry control samples, but comparison with the wet control sample failed to reveal differences that could be attributed to carbon tetrachloride treatment.

From these data it is concluded that prior treatment of damp wheat with carbon tetrachloride for a period of 25 days does not have any effect on the baking quality of flour milled therefrom. It was observed, however, that storage at 24% moisture content lowered the tolerance toward severe treatment with improvers.

In view of Swanson's results (17), published after this work was completed, longer exposure to the carbon tetrachloride should have been made, in order to ascertain whether or not its action is similar to that of Ceresan. Trials involving long exposures are in progress and the results will be reported in a later paper.

References

1. Bailey, C. H. and Gurjar, A. M. J. Agr. Research, 12 : 685-713. 1919.
2. Bakke, A. L. and Noecker, N. L. Iowa State Agr. Exp. Sta. Res. Bull. 165. 1933.
3. Darsie, M. L., Elliott, C. and Peirce, G. J. Bot. Gaz. 58 : 101-136. 1914.
4. Gilman, J. C. and Barron, D. H. Plant Physiol. 5 : 565-575. 1930.
5. Isatschenko, B. L., Ontschukova, M. M., Predtetschenskaja, A. A. and Lipskaja, T. V. Comp. rend. acad. sci. U.S.S.R. 1 : 507-509. 1934.
6. Kolkwitz, R. Ber. deut. botan. Ges. 19 : 285-287. 1901.
7. Larmour, R. K., Geddes, W. F. and Cameron, D. Can. J. Research, 9 : 486-501. 1933.
8. Mead, H. W. Sci. Agr. 13 : 304. 1933.
9. Miehe, H. Die Selbsterhitzung des Heues. Eine biologische Studie (1-127) Jena. 1907.
10. Miehe, H. Arch. Microbiol. 1 : 78-118. 1930.
11. Norman, A. G. Ann. Appl. Biol. 18 : 244-259. 1931.
12. Passerini, N. Boll. 1st. super agrar. Pisa. 8 : 711-741. 1932.
13. Peirce, G. J. Bot. Gaz. 53 : 89-112. 1912.
14. Quam, O. Landmansblad, 23 : 61-64. 1904.
15. Smith, H. J. and Bartz, J. P. Cereal Chem. 9 : 393-401. 1932.
16. Strand, A. L. Univ. of Minn. Agr. Exp. Sta. Tech. Bull. 49. 1927.
17. Swanson, C. O. Cereal Chem. 11 : 173–199. 1934.
18. Tomkins, R. G. Proc. Roy. Soc. Series B. 111: 210-226. 1932.

BLOOD PARASITES OF RUFFED GROUSE (*BONASA UMBELLUS*) AND SPRUCE GROUSE (*CANACHITES CANADENSIS*), WITH DESCRIPTION OF *LEUCOCYTOZOON BONASAE* N. SP.[1]

By C. H. D. Clarke[2]

Abstract

A list of blood parasites found in ruffed and spruce grouse, including *Leucocytozoon bonasae* n. sp., *Trypanosoma gallinarum* Bruce *et al.*, 1911, and Microfilariae, is given. Members of the genus *Leuocytozoon* being known to be pathogenic, the possibility of a connection between *Leucocytozoon bonasae* and the problem of grouse periodicity is suggested.

For the past three years the writer has been engaged in an investigation of the cycle in numbers of grouse, particularly of the ruffed grouse, (*Bonasa umbellus* (L.)), in Ontario. The study of grouse haematozoa has been part of this, and it is felt that the time has come to record the species found.

Leucocytozoon

This parasite was first found at Frank's Bay, Lake Nipissing, Ontario, in May, 1933. Later in 1933 it was discovered at Biggar Lake, Algonquin Park, where young grouse had obviously been decimated. It was especially abundant in a smear from a very sick bird. Attention has already been called (2) to its occurrence at Brule Lake, Algonquin Park, in the summer of 1934, and its association with a heavy mortality of young birds. A similar condition was found at Frank's Bay in the same season. It is not the purpose here to discuss the possible significance of its occurrence beyond recalling that the pathogenicity of certain members of the genus is well established (5, 12, 15, 19).

Description of gametocytes. In the classification of Marcel and André Leger (6) the species under consideration is of the fusiform type, with the host-cell nucleus compact. Micro- or male gametocytes are noticeably less frequent than macro- or female gametocytes and are characterized by taking a much lighter stain, a feature applying to their host cells as well. Their size ranges overlap completely but the microgametocytes appear more commonly in the smaller limits. The cytoplasm of the macrogametocyte is dark blue (Giemsa stain) granular to alveolar, and vacuolated; that of the microgametocyte is pale blue and more uniform. The nucleus of the macrogametocyte stains a pale red, is usually more or less round, in size about 2.5 or 3μ and located anywhere within the parasite; that of the male pale red, oval, centrally located (usually), and $12 \times 5\mu$ in size. Pigment granules are found in both male and female in greatly varying amounts; female cells contain on the average more pigment. The parasites themselves measure 18 to 20μ by 6 to 7μ; often more rounded forms around $12 \times 15\mu$ are found. The host cell is

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10 to 12μ in width and varies in length from 25 to 40μ depending on the attenuate ends. Its nucleus, a dark red body, lies to one side. Its cytoplasm is seen only at the ends. These are characteristically coarsely granular in this species, with red staining chromatoid granules.

No chromatin has been found in the male nucleus, that might be interpreted as a karyosome. In the female nucleus a more or less compact group of red staining granules is often seen. Likewise no scattered chromatin granules have been found. Red staining granules have been noticed but these appear to be identical with the granules in the ends and have not been seen in parasites free from their host cell. Certain gametocytes have been found in which the host-cell nucleus lay either beneath or on top of the parasite. These forms are more attenuated (5 or $6\mu \times 20$ to 25μ, parasite; and 35 to 55μ, host cell), and are considered immature, since they are not present to any extent in mature birds, especially in spring. Developing forms are rare in peripheral blood (9).

In size and general morphology this species most closely resembles *L. lovati* Seligman and Sambon (14) of the red grouse (*Lagopus scoticus*) and *L. mansoni* Sambon (13) of the capercailzie, (*Tetrao urogallus*). It differs in its oval shape and in the reduced length of the fusiform ends of the host cell. References to other species are appended. Obviously the two species above are closely related and the differences may be those of host only. With the limitations due to our absolute lack of knowledge of relationships within this genus, the name *Leucocytozoon bonasae* sp. nov. is proposed for the species under consideration.

Schizogony in *Bonasa umbellus*

Ruffed grouse No. 126, (in the specimens collected during the study of grouse periodicity), adult male, Frank's Bay, May 19, 1933. Also a series from Biggar Lake, July 1933, Frank's Bay, June 1934, and Brule Lake, June, July and August 1934.

Canachites canadensis

A species of *Leucocytozoon*, not sufficiently well preserved for specific determination was found in spruce grouse No. 124, adult female, Frank's Bay, May 15, 1933.

The specimens described and figures are from ruffed grouse No. 185, juvenile male, Brule Lake, August 16, 1934.

Sporogony: unknown.

Organs for the study of schizogony were preserved and are to be studied shortly.

Trypanosomes

Trypanosomes were recorded from ruffed grouse in Michigan by Stafseth and Kotlan (16) in 1925, without further identification or description. In the present study they have been found in small numbers in blood smears from

three grouse. No cultures were made owing to the limitations of a field laboratory. All specimens observed were in the large, S-shaped stage described by Novy and MacNeal (11) as the most mature.

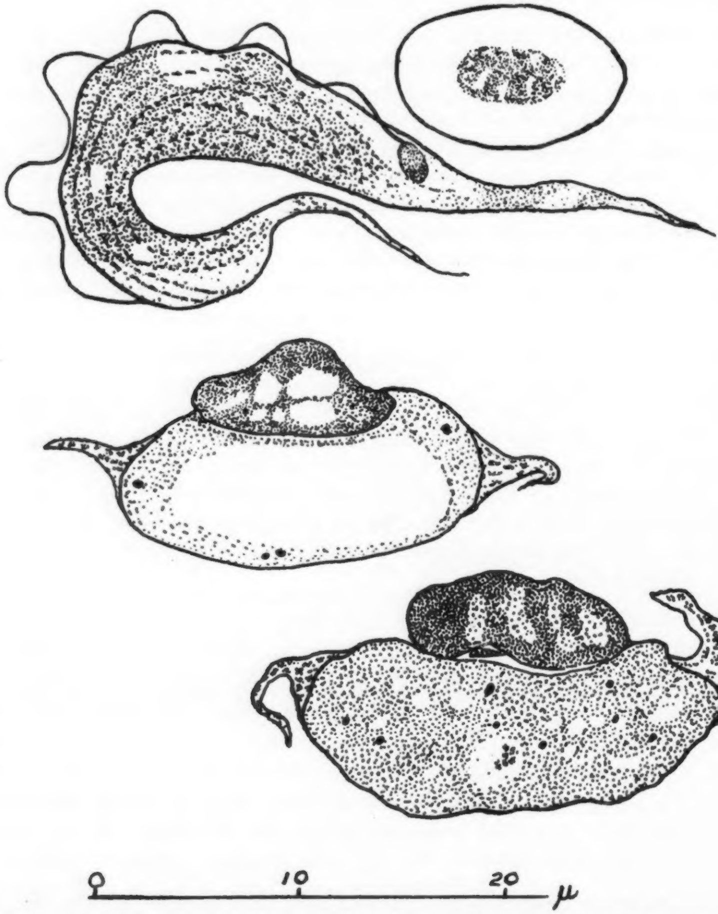


FIG. 1. *Top—Trypanosome of the ruffed grouse. Middle—Leucocytozoon bonasae, microgametocyte. Bottom—Leucocytozoon bonasae, macrogametocyte. Erythrocyte shown for comparison.*

The specimens, which are all curved as they lie on the slide, are about 55μ long by 5μ wide. The ***kinetoplast*** stains a dense red (Giemsa), is oval, $2\mu \times 1\mu$, sometimes smaller, and lies about 15μ from the posterior end. The ***undulating membrane*** is plainly visible. The free flagellum appears to be short, 2μ or so, but this may be due to deficient staining. The ***nucleus*** is roundish, and stains a pale red. It lies on the outside curve of the body

midway between the ends; in diameter about 3μ, it varies with the degree of flattening of the specimen on the slide. The *cytoplasm* stains a dark blue with a few clear areas located in the anterior and posterior ends and around the kinetoplast. The general appearance is granular with some vacuoles. A number of specimens show *myonemes*, the number of lines varying from five to eight according to the flattening of the specimen.

Many trypanosomes are polymorphic. In the absence of cultures and in view of the fewness of specimens the identification here must be considered incomplete. A very narrow range of variation of *Trypanosoma gallinarum* Bruce *et al.* (1), would include all the specimens seen, and to this species it must be temporarily referred.

Host Records

Bonasa umbellus. The specimens described and the figures are from ruffed grouse No. 176, juvenile male, Brule Lake, July 10, 1934.

Other Brule Lake records are No. 168, adult male, June 7, 1934, and No. 169, adult male, June 14, 1934.

Canachites canadensis, spruce grouse No. 124, Frank's Bay, May 15, 1933.

Microfilariae

Microfilariae have been found in two instances, both sets being similar. The adult worms were not found in spite of the most careful dissection. Larvae were not abundant.

Host Records

Ruffed grouse No. 168, adult male, Brule Lake, June 7, 1934.

Spruce grouse No. 124, adult female, Frank's Bay, May 15, 1933.

References

1. Bruce, D., Hamerton, A. E., Bateman, H. R., Mackie, F. P. and Lady Bruce. Rept. Sleeping Sickness, Comm. Roy. Soc. London, 11 : 170-183. 1911.
2. Clarke, C. H. D. Causes of mortality of young grouse. Science, 80 : 228-229. 1934.
3. Fantham, H. B. Observations on the parasitic protozoa of the red grouse (*Lagopus scoticus*) with a note on the grouse fly. Proc. Zool. Soc. London, 2 : 692-708. 1910.
4. Hartman, Ernest. The asexual cycle in *Leucocytozoon anatis*. J. Parasit. 15 : 178-182. 1929.
5. Knuth, P. and Magdeburg, F. Uber ein durch Leukocytozoon verursachtes Sterben junger Gänse. Berl. tierärztl. Wochschr. 38 : 359-361. 1922.
6. Leger, M. and Leger, A. Les Leucocytozoon: leur dénombrement et essai de classification. Bull. soc. path. exot. 437-447. 1914.
7. Mathis, C. and Leger, M. Trypanosome de la poule. Compt. rend. soc. biol. (Paris), 67 : 452-454. 1909.
8. Mathis, C. and Leger, M. Leucocytozoon de la poule. Compt. rend. soc. biol. (Paris), 67 : 470-472. 1909.
9. Mathis, C. and Leger, M. Récherches sur le Leucocytozoon de la poule. Périodicité des formes sexuées dans le sang. Compt. rend. soc. biol. (Paris), 67 : 688-690. 1909.
10. Mathis, C. and Leger, M. Sur un nouveau Leucocytozoon de la poule. Compt. rend. soc. biol. (Paris), 68 : 22-24. 1910.
11. Novy, F. G. and MacNeal, W. J. On the Trypanosomes of birds. J. Infectious Diseases, 2 : 256-308. 1905.

12. O'Roke, E. C. A malaria-like disease of ducks. Univ. Mich. School of Forestry and Conservation, Bull. 4. 1934.

13. Sambon, L. W. Remarks on the avian hemoprotozoa of the genus *Leucocytozoon* Danilewsky. J. Trop. Med. 11 : 245-248; 325-328. 1908.

14. Seligman, C. G. and Sambon, L. W. Preliminary note on a Leucocytozoon found in the blood of red grouse (*Lagopus scoticus*). Lancet, p. 829. Sept. 21, 1907.

15. Skidmore, Louis V. *Leucocytozoon smithi*. Infection in turkeys and its transmission by *Simulium occidentale* (Townsend). Zentr. Bakt., Parasitenk. Infekt. Abt. 1. Orig. B. 125 : 329-335. 1932.

16. Stafseth, H. J. and Kotlan, A. Report of investigations on an alleged epizootic of ruffed grouse in Michigan. J. Am. Vet. Med. Assoc. 67 : 260-267. 1925.

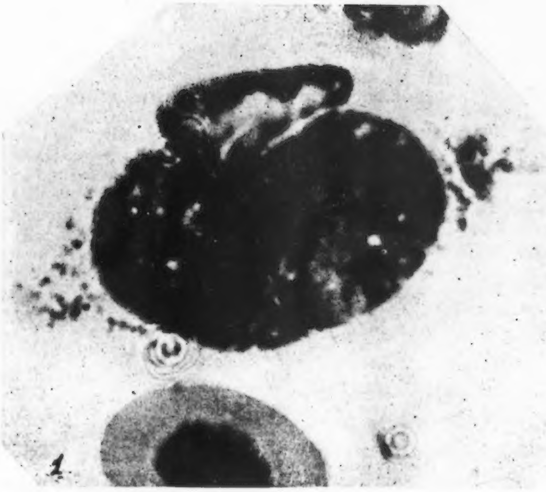
17. Vassal, J. J. Sur un nouveau Trypanosome aviaire. Compt. rend. soc. biol. 58 : 1014-1015. 1905.

18. Volkmar, Fritz. Observations on *Leucocytozoon smithi;* with notes on Leucocytozoa in other poultry. J. Parasit. 16 : 24-28. 1929.

19. Walker, J. A short note on the occurrence of a Leucocytozoon infection; host, the ostrich. Trans Roy. Soc. South Africa, 3 : 35-38. 1913.

20. Wenyon, C. M. Protozoology. 2 vol. Baillière, Tindall and Cox, London. 1926.

PLATE I



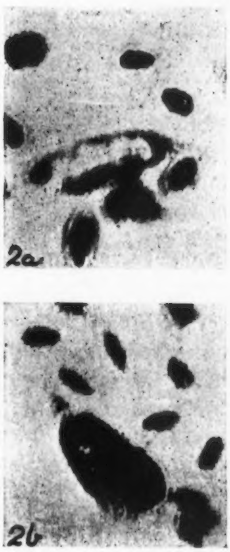


FIG. 1. *Leucocytozoon bonasae. Macrogametocyte.* FIG. 2. *Leucocytozoon bonasae. a. microgametocyte. b. macrogametocyte. Both taken from the same field of photograph.* FIG. 3. *Trypanosome of the ruffed grouse.*

VARIATION IN WEIGHT OF SOME INTERNAL ORGANS OF THE DOMESTIC FOWL *(GALLUS GALLUS)*[1]

By J. W. Hopkins[2] and J. Biely[3]

Abstract

One hundred apparently normal yearling single-comb White Leghorn hens were subjected to post mortem examination, including weighing of the liver, kidneys and spleen. The average weight of these organs (excluding two individuals possessing only one kidney) constituted 1.89, 0.64 and 0.12% of the average total live weight. Single organs, particularly the spleen, were relatively more variable than total body weight.

The weight of the organs from individuals of similar total live weight varied markedly, but, on the whole, the larger birds had somewhat larger organs. As the size of bird increased, however, the average percentage of the total weight due to liver, kidneys or spleen decreased.

There was a moderate but significant correlation ($r = +0.41$) between the weights of liver and kidneys from birds of a specified total weight, but no evidence of association between weight of kidneys and spleen weight.

Introduction

In the course of a previously reported study of normal and pullorum-disease-infected fowls (1), 100 apparently normal yearling single-comb White Leghorn hens were subjected to post mortem examination, including weighing of the liver, kidneys and spleen. A statistical study has been made of the recorded weights of these organs, both individually and in relation to the weight of the bird as a whole.

Previous quantitative anatomical investigations of the fowl seem to have been concerned chiefly with tracing the relative growth of the various systems and organs. Thus Latimer (2) found that the average weight of liver and kidneys, relative to that of the body as a whole, decreased from early maxima of 6.2 and 2% to approximately 2.5 and 0.6% in the adult single-comb White Leghorn (including both sexes). The actual weight of the liver increased noticeably in the older birds however, especially in the fat hens. The relative weight of the spleen (3) also decreased with age, from an early maximum. Analogous results are reported by Mitchell, Card and Hamilton (4) from successive groups of five White Plymouth Rock cockerels, pullets and capons selected from a large flock. As the approximate slaughter weight of cockerels increased from one to seven pounds, the percentage of that weight due to liver, kidneys and spleen decreased from 3.7, 1.1 and 0.21 to 1.3, 0.39 and 0.11 respectively. The liver, kidneys and spleen of approximately 5-lb. pullets formed on the average 1.9, 0.62 and 0.21% of the total bird weight.

[1] *Manuscript received February 12, 1935.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Department of Poultry Husbandry, University of British Columbia, Vancouver, B.C.
[2] *Biologist, National Research Laboratories.*
[3] *Research Assistant, Department of Poultry Husbandry, University of British Columbia.*

In both of the foregoing investigations the size of the organs of individual birds of the same age or weight varied considerably, but no quantitative study of such differences was made. Souba (5) on the other hand specifically investigated this variation, using single-comb White Leghorn cockerels 100 to 120 days of age, *i.e.*, in the phase of rapid growth associated with puberty. The coefficient of variation of total body weight proved to be 19.0, whereas the coefficients of variation of the kidneys, liver and spleen were 19.2, 21.9 and 35.9 respectively. There was also a highly significant correlation between total bird weight and the weight of individual organs. These results, as mentioned, were obtained from actively growing birds. The authors are not aware of any similar biometric data respecting mature fowls.

Material and Methods

The 100 yearling hens furnishing the anatomical data were secured from several commercial flocks. They were kept together in one house, under uniform conditions of feeding and management, for about one month prior to slaughter, and throughout this period of observation all appeared to be healthy and vigorous. Only a few were laying; about 25 were in moult, and the remainder were just beginning to shed their feathers.

Killings were carried out between October 18 and November 4, 1933, at which time the birds were approximately 17 to 18 months old. Those selected for slaughter on any given day were transferred from the laying house to exhibition cages, containing food and water, in the laboratory. Immediately prior to killing, all birds were weighed, the live weight including the food present in the digestive tract. Whilst the body was still warm, the liver, spleen and kidneys were cut out in the order named and weighed as rapidly as possible, in order to minimize losses due to evaporation. The gall bladder was removed from the liver, and the ureters and excess fat from the kidneys; both kidneys were weighed together. The weights obtained are shown in Table I.

As stated, a post mortem examination was made of every carcass. The internal organs appeared to be normal in every respect save that two birds had one kidney only, a condition not previously observed in any of the more than 1000 specimens examined in this laboratory. These were excluded from subsequent calculations. Only a few cases of infestation of the intestine with roundworms or tapeworms were observed, and in no instance did these parasites appear to be present in sufficiently large numbers to affect general health. The birds may therefore be regarded as comprising a group representative of flocks kept in good health and free from parasitic or infectious disease.

Variation in Weight of Bird and Organs

The total live weight and the weight of the organs of the 98 individual birds are shown graphically in Fig. 1. Total weights recorded vary from 1200 to 2175 gm., the average being 1590.7 gm. Individual livers fall between 21.5 and 52.3 gm., with an average of 30.00 gm.; kidneys between 6.9 and

TABLE I

LIVE WEIGHT AND WEIGHT OF INTERNAL ORGANS OF HENS

Total live weight, gm.	Weight of liver, gm.	Weight of kidneys, gm.	Weight of spleen, gm.	Total live weight, gm.	Weight of liver, gm.	Weight of kidneys, gm.	Weight of spleen, gm.
1200	26.9	8.06	2.01	1591	35.6	9.96	1.32
1212	29.4	9.08	2.10	1593	32.4	14.60	1.70
1216	24.1	8.11	1.14	1594	41.5	11.74	1.67
1230	29.5	8.15	2.31	1600	31.7	13.83	1.34
1272	24.7	9.58	1.55	1600	29.7	7.80	2.35
1307	26.3	10.37	1.34	1615	27.0	10.38	1.68
1320	30.0	8.14	2.66	1616	25.2	8.62	1.67
1328	37.7	10.92	1.42	1618	27.7	10.12	1.90
1340	22.6	7.45	1.16	1620	40.3	11.45	1.74
1340	28.2	8.52	2.88	1635	32.3	10.06	2.60
1372	26.0	7.02	1.04	1640	29.0	8.93	1.20
1372	26.3	7.41*	1.62	1645	25.9	10.08	1.26
1375	22.8	8.57	1.19	1654	25.8	10.52	1.28
1380	30.0	8.35	2.88	1658	23.8	9.14	1.02
1390	28.3	11.00	1.27	1664	28.2	8.27	2.12
1400	23.1	9.46	1.29	1669	34.6	9.99	3.40
1403	26.1	8.52	2.63	1672	27.3	8.55	1.46
1404	27.5	11.51	2.34	1674	39.1	8.30	2.06
1412	28.3	10.14	1.84	1675	44.7	13.01	1.97
1415	22.9	8.99	1.52	1680	32.2	11.60	1.28
1416	39.6	10.84	1.68	1684	34.9	10.94	2.24
1417	29.5	9.35	1.07	1690	24.9	6.91	1.48
1420	23.4	10.22	1.20	1715	32.5	10.82	1.94
1430	27.3	10.37	1.88	1727	29.8	11.67	1.53
1430	26.6	9.08	1.87	1728	32.8	14.07	2.16
1436	29.3	10.48	1.35	1730	27.4	10.18	2.10
1438	33.3	11.58	4.26	1730	32.2	12.29	1.90
1445	36.2	10.99	2.61	1735	25.6	10.61	1.33
1450	29.3	9.80	1.87	1735	26.4	9.03	1.88
1454	31.0	8.88	1.14	1739	31.7	9.14	1.21
1455	32.3	8.26	1.48	1740	22.8	11.22	2.39
1455	41.2	14.54	0.90	1748	38.7	11.04	3.14
1460	28.3	8.66	1.28	1750	28.1	9.55	1.51
1461	35.0	12.69	1.49	1777	27.4	10.26	2.03
1496	23.4	9.53	2.37	1782	29.8	13.17	2.18
1505	31.6	8.46	2.14	1787	27.6	10.39	1.60
1512	25.9	9.23	1.67	1788	34.1	9.09	2.68
1518	26.7	10.28†	1.47	1789	25.2	7.46	1.33
1520	28.9	9.68	1.18	1800	37.5	13.18	1.79
1530	30.9	10.54	1.05	1830	27.5	10.86	2.68
1544	30.4	8.11	1.48	1831	35.2	12.02	1.93
1550	29.8	9.45	1.62	1844	29.5	8.39	1.70
1557	34.1	14.60	1.94	1851	29.8	11.44	3.02
1559	27.3	8.39	1.46	1880	35.2	13.38	2.90
1566	25.5	10.02	1.98	1900	28.5	10.62	1.93
1570	31.2	9.38	3.10	1903	27.2	10.12	2.50
1573	26.0	10.32	3.68	1960	31.8	11.45	2.18
1583	30.6	8.35	1.33	2000	29.2	11.05	2.84
1587	30.6	14.54	1.65	2100	36.1	11.08	3.12
1591	21.5	11.52	1.58	2175	52.3	13.26	1.74

* *Left kidney only; no right one.*

† *Right kidney only; no left one.*

14.6 gm., with an average of 10.22 gm.; and spleens between 0.90 and 4.26, with an average of 1.886 gm. The average liver, kidney and spleen weights constitute 1.89, 0.64 and 0.12% of the average total live weight. The standard deviation of total live weight was found to be 195.0 gm., or 12.3% of the mean; and that of the liver, kidneys and spleen to be 5.27, 1.79 and 0.65 gm. or 17.6, 17.6 and 34.5% of the mean respectively. Single organs,

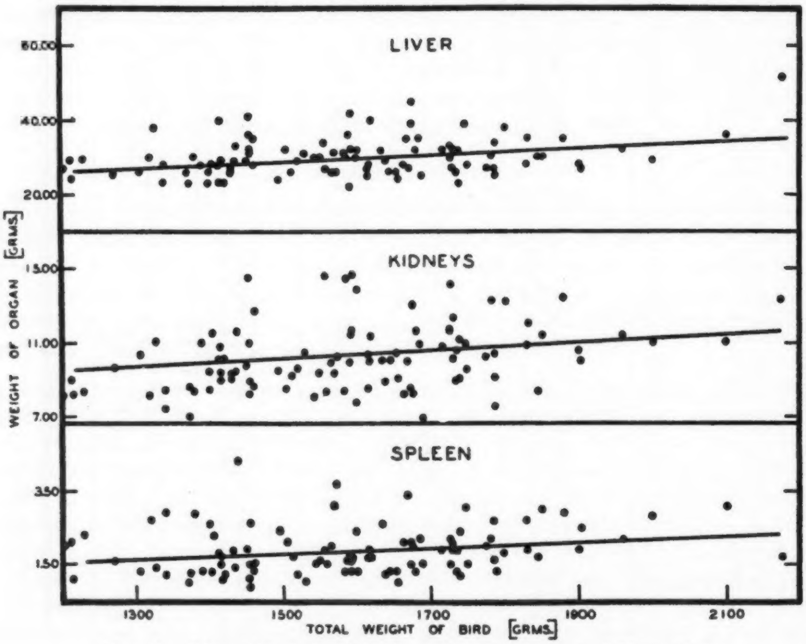


FIG. 1. *Live weight and weight of certain internal organs of 98 yearling single-comb White Leghorn hens.*

and particularly the spleen, are thus relatively more variable in weight than the body as a whole, as in the case of the growing cockerels studied by Souba (5). It might be thought that the high relative variability of the observed spleen weights was occasioned by operational injuries or small amounts of adhering tissue exerting an appreciable influence on the total weight of so small an organ; but in fact the spleen was the easiest of the three organs to excise, as it is encapsulated and can be removed as a whole. The method of killing (breaking the neck) may however have resulted in some of the spleens being more engorged with blood than others.

It will be evident from Fig. 1 that there is a marked variation in the weight of, for example, the liver from birds of similar total weight; and this is also true of the kidneys and spleen. Nevertheless the size of these organs is not wholly independent of the size of the bird. Computation of the coefficient of correlation between the total weight and liver, kidney and spleen weight

yields values of $r = +0.32$, $+0.34$ and $+0.22$ respectively. Although certainly not indicative of any high degree of association, the lowest of these values exceeds the 5% point (0.20) and the other two exceed the 1% point (0.26) for a sample of the size considered, and it may be concluded that there is a tendency for the larger birds to have on the average larger organs. The correlation is, however, much more moderate than that encountered by Souba (5), possibly because differences in the gross weight of mature hens are due not only to differences in the development of the individual as a whole, but also to variations in the amount of muscular tissue or fat and particularly in the condition of the ovary and oviduct from bird to bird, which have probably little relation to organ development.

The regression equations, giving the average organ weight associated with any specified bird weight, in gm., are:

$$\begin{aligned} \text{Liver wt.} &= 16.34 + 0.00859 \text{ total wt.} \\ \text{Kidney wt.} &= 5.19 + 0.00316 \text{ total wt.} \\ \text{Spleen wt.} &= 0.718 + 0.000734 \text{ total wt.} \end{aligned}$$

The course of these functions is illustrated by the regression lines in Fig. 1. All are of the form $y = a + bx$, where a is a positive quantity; the ratio y/x, that is of any organ weight to total weight, will therefore diminish with increasing total weight. This is illustrated in Table II, in which the average organ weights of 1200-, 1700- and 2200-gm. birds, calculated from the foregoing formulas, are expressed as a percentage of the respective total live weights. It is interesting to observe this tendency in individual fowls of the same age group, as well as in the averages of different age groups dealt with by the investigators previously mentioned.

TABLE II

WEIGHT OF ORGANS AS PERCENTAGE OF TOTAL LIVE WEIGHT

Total live weight, gm.	Liver, %	Kidneys, %	Spleen, %
1200	2.22	0.75	0.13
1700	1.82	0.62	0.12
2200	1.60	0.55	0.11

The regression equations may also be used to compute the standard deviation of the individual organ weights from the appropriate regression line. In this way, allowance is made for the increase in average organ weight with total live weight, and an estimate of the variation in weight of the organs of birds of any specified total weight is provided. Owing to the low degree of correlation these adjusted values differ but little from those neglecting the regression. They are 5.02, 1.69 and 0.638 gm., or 16.7, 16.6 and 33.8% of the mean in the case of liver, kidneys and spleen respectively.

Correlation between the weight of different organs from the same bird may also be considered briefly. Neglecting the regression on total body weight, the correlation coefficient between liver weight and weight of kidneys is found to be $+0.48$, and that between weight of kidneys and spleen weight to be $+0.11$. A part of this association however is due to the fact that on the

average all the organs considered tend to be larger in the larger birds. When due allowance is made for the influence of total weight, the above coefficients are reduced to +0.41 and +0.03 respectively. There would thus appear to be a moderate but significant correlation between the weight of liver and kidneys from birds of a specified total weight, but no evidence of association between weight of kidneys and spleen weight.

References

1. Biely, J. and Roach, W. A comparison of the routine rapid whole blood (stained antigen) and the routine rapid serum agglutination tests for pullorum disease. Can. J. Research, 10 : 798-806. 1934.
2. Latimer, H. B. Postnatal growth of the body, systems, and organs of the single-comb White Leghorn chicken. J. Agr. Research, 29 : 363-397. 1924.
3. Latimer, H. B. The relative postnatal growth of the systems and organs of the chicken. Anat. Record, 31 : 233-253. 1925.
4. Mitchell, H. H., Card, L. E., and Hamilton, T. S. The growth of White Plymouth Rock chickens. Illinois Sta. Bull. 278. 1926.
5. Souba, A. J. Variation and correlations of the organs of single comb White Leghorn cockerels. Anat. Record, 26 : 291-297. 1923.

THE HETEROTHALLISM OF *PANAEOLUS SUBBALTEATUS* BERK., A SCLEROTIUM-PRODUCING AGARIC[1]

By Harold J. Brodie[2]

Abstract

Panaeolus subbalteatus, an uncommon coprophilous agaric, has been grown in single-spore culture on malt-extract agar.

The fungus is heterothallic, exhibiting four sexual groups and a remarkable regularity in its pairing reactions.

Both haplophytes and diplophytes produce sclerotia of a striking greenish-blue color. These sclerotia are capable of producing mycelium, even after they have been dried for some weeks, but do not give rise to fruit bodies.

The haplophyte is distinguishable macroscopically as well as microscopically from the diplophyte.

The mycelia do not exhibit the phenomenon of mutual aversion or *barrage*.

Panaeolus subbalteatus Berk. is a rather uncommon coprophilous agaric which may be found in gardens and on lawns where manure has been used to enrich the soil. A description of the species has been given by A. H. Smith (3) who was the first to record its occurrence in the state of Michigan. The fungus was found on the campus of the University of Michigan at Ann Arbor in October 1933. It was identified by Dr. Smith who kindly gave several specimens to the writer.

From a deposit of basidiospores shed by a single fruit body, a series of 25 monosporous cultures was obtained by the sprayed-plate method (2). The black apiculate basidiospores (measuring $8 \times 12\ \mu$) germinated in 12 hr. on malt-extract agar. The haploid mycelia developed quite slowly, requiring two weeks to grow from the centre to the periphery of an agar plate 9 cm. in diameter.

Owing to the pressure of culture work with other species of agarics, the writer was unable to give further attention to the *Panaeolus* until the month of January. The haploid mycelia were kept in a condition of vigorous growth in the interim by making transfers once every month.

One of the most striking characters of the mycelium of *Panaeolus subbalteatus* in laboratory culture, is the abundant production of sclerotia. A mycelium freshly transferred to a Petri dish of malt agar or of other medium grows for about five days, producing only ordinary vegetative hyphae. The mycelium then becomes laden with numerous large drops of watery exudate and at the same time small masses of mycelium begin to accumulate on the older portions of the thallus. The sclerotial primordia are of a striking greenish-blue color. Upon discovering nodules of bright greenish-blue lying upon the white mycelium, in all the culture tubes, the writer's first impression was that the entire stock had become contaminated with a *Penicillium*. Closer examination revealed the fact that the nodules were in reality sclerotia,

[1] *Manuscript received January 19, 1935.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada.
[2] *Fellow of the National Research Council (United States) at the University of Toronto.*

spherical in shape and varying in size from 1 to 4 mm. with an occasional one as much as 6 mm. in diameter. Plate I, Fig. 2, shows the appearance of the sclerotia.

Cultures varied in the tendency which they exhibited to produce sclerotia. Occasionally only a certain sector of a culture on an agar plate would produce sclerotia, the remainder of the mycelium being fluffy and devoid of them. Such a culture is shown in Plate I, Fig. 4, *B*.

The sclerotia soon became hard and darker in color. Some were allowed to dry for a month and when these were placed on a freshly poured agar plate they germinated to give rise to haploid mycelium. Frequently sclerotia are known to give rise to fruit bodies but the writer was able to obtain only vegetative mycelium by allowing the sclerotia to "germinate".

On January 9, 1934, the haploid mycelia were paired in tubes in all possible combinations and two weeks later the pairings were examined. In some tubes diploid mycelium had made its appearance and could be distinguished from the haploid mycelium macroscopically. The hyphae of the diploid mycelium are slightly straighter and coarser than the hyphae of the haploid mycelium. The diploid mycelium not only appeared along the line of contact of the two haplophytes but also developed from the haploid mycelium, indicating that in *Panaeolus subbalteatus* diploidization is similar to what has been described by Buller (1) for *Coprinus lagopus*.

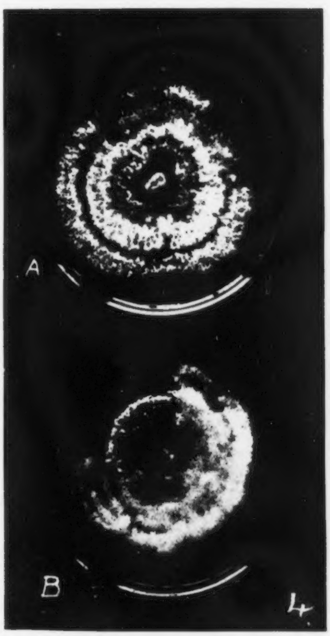
The haploid mycelium is composed of fine hyphae whose average diameter is 4 μ. The angle of branching tends to be rather wide. The hyphae of the diploid mycelium are coarse (about 5–6 μ in diameter) and branch at an angle considerably smaller than that of the haploid mycelium. Although the haplophyte grows somewhat faster than the diplophyte, the difference in growth rate is not great and was not measured. The clamp connections on the diploid mycelium are small, the hook cell being much less arched than in some fungi such as *Coprinus lagopus* (1, p. 202). The two kinds of mycelium are contrasted in Text-fig. 1, *A* and *B*.

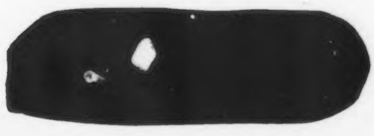
TEXT-FIG. 1. *The haploid (A) and diploid (B) mycelia of Panaeolus subbalteatus, each growing in a hanging drop of malt-extract agar. Magnification, 90.*

Sclerotia appeared on all diploid as well as haploid mycelia. The sclerotia occurring on the diplophytes gave rise to diploid mycelium and never to haploid.

PLATE I

1





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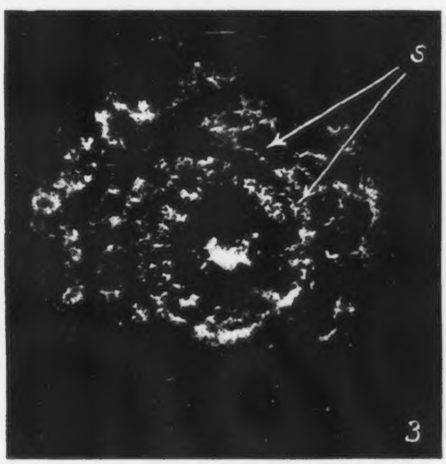

Panaeolus subbalteatus. FIG. 1. *Specimens collected by Dr. A. H. Smith on the campus of the University of Michigan, June 1934, and photographed by him. The specimens are smaller than some collected in 1933 and probably represent a dry weather form of the fungus. Natural size.* FIG. 2. *Agar and haploid mycelium withdrawn from culture tube, showing sclerotia. Natural size.* FIG. 3. *A diploid mycelium three weeks old showing the sclerotia, s. Two-thirds natural size.* FIG. 4. *B, a haploid mycelium, No. 2 (Ab), two weeks after having been planted on an agar plate. One sector only of the mycelium is producing sclerotia which are seen as a dense white arc half-way between the centre and the periphery of the mycelial mat; A, a diploid mycelium, No. 2 + 6, the same age as the haplophyte and also producing sclerotia. One-half natural size.*

Neither haplophyte nor diplophyte showed any tendency to produce carpophores in culture, nor did either produce the oidia which are so frequently found in hymenomycetous fungi.

In Text-fig. 2 the results of the pairings are given in tabular form, a plus sign indicating the presence of clamp connections and a minus sign their absence. In the long series of pairings there were no irregularities, the mycelia falling perfectly into four groups.

		AB						*ab*							*Ab*					*aB*						
		1	*4*	*10*	*11*	*20*	*25*	*5*	*8*	*9*	*13*	*17*	*19*	*21*	*2*	*3*	*7*	*12*	*15*	*6*	*14*	*16*	*18*	*22*	*23*	*24*
AB	*1*		−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	4	−		−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	10	−	−		−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	11	−	−	−		−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	20	−	−	−	−		−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	25	−	−	−	−	−		+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
ab	*5*	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	13	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	17	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
	19	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−
	21	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−
Ab	*2*	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	+	+	+	+	+	+	+
	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+
aB	*6*	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+		−	−	−	−	−	−
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−		−	−	−	−	−
	16	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−		−	−	−	−
	18	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−		−	−	−
	22	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−		−	−
	23	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−		−
	24	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	

TEXT-FIG. 2. *The heterothallism of Panaeolus subbalteatus demonstrated by pairing monosporous mycelia in all possible combinations.*

In none of the pairings between haploid mycelia of *Panaeolus subbalteatus* was there exhibited the phenomenon of aversion or "barrage" which has been described by Vandendries and Brodie (7) for *Lenzites betulina.*

From the results of the pairing experiment we may conclude that *Panaeolus subbalteatus* is heterothallic and that it exhibits four sexual groups*. As far as the writer is aware, the sexuality of only two other species of *Panaeolus*

**For possible future reference, cultures of the haploid mycelia Nos. 1 (AB), 5 (ab), 2 (Ab) and 6 (aB) have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland, as well as the two diploid mycelia Nos. 1 + 5 and 2 + 6.*

has been ascertained. *P. campanulatus* Fr., was shown to be tetrapolar in 1923 by Vandendries (4) and the same investigator later (5) reported *P. papilionaceus* Fr., as being tetrapolar. Both of these species exhibit in some degree the phenomenon of "barrage" or repulsion between certain mycelia. Vandendries (6), reporting on his attempts to obtain the germination of spores of various species of *Panaeolus*, lists the following four as germinable: *P. campanulatus*, *P. fimicola*, *P. separatus* and *P. papilionaceus*. He was unable to germinate the spores of *P. sphinctrinus*.

In concluding, the writer wishes to express his gratitude to Dr. A. H. Smith for the gift of material from which the research recorded above was made and for notes regarding the species.

References

1. Buller, A. H. R. Researches on fungi, 4 : 204-213. 1931.
2. Lohman, Marion L. Pap. Mich. Acad. Sci. Arts and Letters, 13 : 141-157. 1931.
3. Smith, A. H. New and unusual Agarics from Michigan. Ann. Mycologici, 32 : 471-484. 1934.
4. Vandendries, René. Mém. in 4° de l'Acad. roy. de Belgique, 1923.
5. Vandendries, René. Mém. spéç. du Mus. d'hist. nat. Paris, Sept. 1931.
6. Vandendries, René. Bull. Soc. Myc. de France, 49 : 130-165. 1933.
7. Vandendries, René et Brodie, Harold J. La Cellule, 42 : 165-209. 1933.

THE OIDIA OF *PSILOCYBE COPROPHILA* AND THE PAIRING REACTIONS OF MONOSPOROUS MYCELIA[1]

BY HAROLD J. BRODIE[2]

Abstract

By isolating thirty monosporous mycelia and pairing them, the agaric *Psilocybe coprophila* Fr. has been shown to be *heterothallic* and bipolar, confirming the report of Miss Kathryn Gilmore (1926). Contrary to the statement of Miss Gilmore, no oidia were found on the diploid mycelia although they are abundant on the haplophytes. The diplophytes are frequently "impure", hyphae devoid of clamp connections and are found intermingled with hyphae bearing clamp connections. The possibility of the clamp-connection-free hyphae being haploid and of the development of oidia on these haploid hyphae is suggested as an explanation of the statement of Miss Gilmore that oidia occur on the diplophyte of this fungus.

No mutual repulsion between haploid mycelia was observed. This is negative evidence in support of the prediction of Vandendries and Brodie that their *barrage* phenomenon would not be found in bipolar but only in tetrapolar hymenomycetes.

Introduction

An interesting account of her study of *Psilocybe coprophila* in culture was published in 1926 by Miss Kathryn Gilmore (5). In connection with researches concerning the function of the oidia of the Hymenomycetes, several points in Miss Gilmore's paper were of particular interest to the writer when he first read it in 1930.

A rather extensive study of the life history and sexuality of *Coprinus lagopus* was being carried on at that time in the laboratory of Professor A. H. R. Buller at Winnipeg. While working with Dr. Buller, the writer (1, 2) studied the function of the oidia of *Coprinus lagopus*. It was shown that: (i) the oidia are produced in little masses in drops which crown the ends of aerial oidiophores; (ii) the oidia are borne on haploid but never on diploid mycelia; (iii) insects can transfer oidia of one sexual strain to mycelia derived from spores of the opposite sexual strain; and (iv) the oidia so transferred may germinate, fuse with the mycelia to which they have been brought, and cause these to become diploid. The conclusion that only the haploid mycelia of the heterothallic hymenomycetous fungi produce oidia seemed to be strengthened by an examination of several other species of agarics: diploid mycelia bearing oidia did not come under observation.

Miss Gilmore stated that, in *Psilocybe coprophila*, oidia are developed not only on the haploid mycelia but also on the diploid. The oidia produced by the haplophytes were found to germinate and give rise to haploid mycelia of the same constitution as the parent culture. But with regard to the oidia developed on a diploid mycelium (produced by pairing two haploid mycelia

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of known sexual strain), Miss Gilmore concluded that: "They sometimes germinate to form diploid mycelia; at other times they germinate to form haploid mycelia, segregation of the nuclei occurring before the oidia are produced." Of seventeen single-oidium cultures, isolated from a diploid mycelium, sixteen were haploid; only one was diploid. Concerning this one Miss Gilmore wrote: "It might have originated from a small bit of diploid mycelium or two unlike oidia erroneously taken for a single germinating oidium."

Because Miss Gilmore had reported finding oidia on the diplophytes of *Psilocybe coprophila* whereas the writer found no oidia on the diplophytes of *Coprinus lagopus* and other *Coprini* and, further, because of some uncertainty in Miss Gilmore's conclusions it seemed advisable to make a fresh study of cultures of the *Psilocybe*. Unfortunately Miss Gilmore's cultures were no longer available and it was not until recently that an opportunity for continuing the study presented itself.

In the meantime the writer (3) examined more than a dozen species of agarics in the genera *Collybia*, *Coprinus*, *Corticium*, *Polystictus*, *Hypholoma* and *Lenzites*. From this research the following conclusions were drawn: (i) that heterothallic hymenomycetes as a rule produce oidia on the haploid mycelium but not on the diploid: and (ii) that, as far as is known, homothallic hymenomycetes do not produce oidia.

An extensive study of *Collybia velutipes* (3) has revealed the fact that oidia are developed on the diplophyte as well as on the haplophyte in this species. These oidia are *haploid* and are borne on haploid branches arising from the diploid mycelium. Such haploid branches are produced by the separation of the nuclei of the dicaryon either by the migration of the nuclei into different branches or, rarely, by the abnormal elongation of the hook cell during the process of the formation of clamp connections. Half the oidia borne on the diplophyte are thus of the same sex as one of the parent mycelia originally used to obtain the diplophyte and half are of the same sex as the other parent mycelium.

Examining Miss Gilmore's results in the light of the above discovery it appeared possible that discounting certain errors (*e.g.*, two unlike oidia erroneously taken for a single oidium), the situation in *Psilocybe coprophila* might fall into line with that in *Collybia velutipes*, *i.e.*, that segregation of the nuclei occurs before the oidia are produced.

That true diploid oidia occur on the diplophytes of *Pholiota aurivella* Batsch., was clearly demonstrated in 1932 by Vandendries and Martens. This species alone, of over twenty referred to in the literature (3) is definitely known to produce diploid oidia. It appears that oidia are most commonly associated with the haploid mycelia of heterothallic species.

There were, therefore, several reasons why a fresh study of *Psilocybe coprophila* might be profitable It was necessary to ascertain: (i) whether or not oidia actually do occur on the diploid mycelium of *P. coprophila;* and (ii) whether the oidia, if present, are haploid or diploid.

There was still another reason for the advisability of examining cultures of *P. coprophila.* In 1933 (7), in collaboration with Dr. René Vandendries, the writer described the phenomenon of mutual repulsion between certain haploid strains of *Lenzites betulina, e.g.*, between (*ab*) and (*aB*) or between (*Ab*) and (*AB*). The repulsion results in a zone barren of mycelium between the two haplophytes which have been paired. The phenomenon has been given the name *barrage.* In defining the term, Vandendries and Brodie declared that repulsion is manifest only in tetrapolar species when the *b* factor is possessed in common by the two haplophytes and the *a* factor differs in each. *A priori* there should be no mutual aversion between haplophytes of a bipolar species where only one pair of factors regulates the pairing of mycelia. *Psilocybe coprophila* had been shown by Miss Gilmore to be bipolar and it appeared to be an excellent subject for testing the correctness of the assumption that "barrage" is not manifest in a bipolar fungus.

The Haploid Mycelia

Several carpophores found on cow dung in Ann Arbor, Michigan, were identified by Dr. A. H. Smith as *Psilocybe coprophila* Fr. (in the sense of Ricken). Dr. Smith kindly gave the specimens to the writer and 30 monosporous cultures were obtained from the spores shed by a single fruit body. The sprayed-plate method of Kauffman (6) was used in isolating the spores.

The mycelia were grown on malt-extract agar (15 gm. malt extract of Merck & Co., and 15 gm. agar to one litre of distilled water), and on the "*IIa*" agar recommended by Miss Gilmore (5, p. 421). The latter precaution was taken to ensure conditions identical with those under which Miss Gilmore grew her cultures. Growth was good on both media, the mycelia forming dense white mats (Plate I, Fig. 1). All the cultures on malt-extract agar possessed a very characteristic odor resembling the odor of sweet corn when cooking. Some of the cultures produced dark brown sclerotia about 4 mm. in diameter and on many of the haplophytes there appeared rudiments of fruit bodies which, however, did not develop into perfect fruit bodies.

All haplophytes produced oidia abundantly. Special coiled hyphae by segmentation give rise to the allantoid oidia which are about 1.5 μ in diameter and 4–8 μ in length.

Miss Gilmore reported (5, p. 423) that of 24 single-spore cultures which she obtained, 23 were haploid and one diploid. All the monosporous mycelia isolated in the present study were haploid, none of the mycelia bearing clamp connections.

The Pairing of Monosporous Mycelia

When the haplophytes were a month old they were paired in tubes in all possible combinations, 436 pairings in all having been made. Clamp connections appeared on some of the mycelia two days after the pairing but the entire series was not examined critically until the end of the second week.

In Text-fig 1 are presented the results of the experiment. In this table a plus sign indicates the presence of clamp connections on the paired mycelia, a minus sign their absence.

		A																	a												
		1	4	5	6	11	12	13	14	15	18	19	21	22	23	24	25	30	2	3	7	8	9	10	16	17	20	26	27	28	29
	1		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	11	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	13	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
A	15	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	18	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	−	−	−	−	−	−	−	−	−	−		−	−	−	−	⊖	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	21	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	22	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	23	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	24	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	25	−	−	−	−	−	−	−	−	−	−	⊖	−	−	−	−		−	+	+	+	+	+	+	+	+	+	+	+	+	+
	30	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−
a	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−		−	−	−	−	−
	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−		−	−	−	−
	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−		−	−	−
	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−		−	−
	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−		−
	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	

TEXT-FIG. 1. *Table showing the results of pairing in all possible combinations of 30 monosporous mycelia of Psilocybe coprophila.*

It will be seen that the mycelia fall into two sexual groups, seventeen in one group and thirteen in the other. The fungus is therefore clearly bipolar as has been shown by Miss Gilmore.

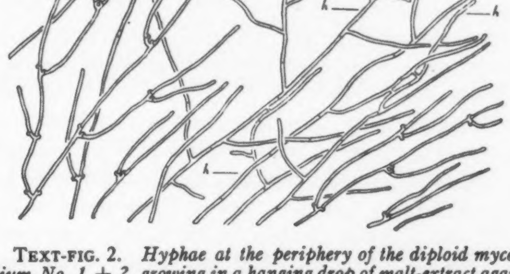
In none of the 435 pairings was there any sign of mutual repulsion between the haploid mycelia. In a few tubes there were lines of demarcation between the individual mycelia but these lines disappeared as intermingling of the mycelia became complete. Inasmuch as not all tetrapolar species of the Hymenomycetes exhibit the barrage phenomenon, its nonappearance in the present example of a bipolar species is only negative evidence in support of the statement of Vandendries and Brodie that barrage is not manifest in a bipolar hymenomycete.

In the table of pairings (Text-fig. 1), the reaction of mycelium No. 19 with No. 25 is indicated as exceptional by means of a circle around the minus sign. The mycelium resulting from the pairing bore a few false clamp connections. However when a confirmatory pairing was made a few days later, the irregularity did not reappear. The cause of the temporary irregularity is unknown.

The Diploid Mycelium

In none of the 221 diplophytes were any oidia to be found, a result which fails to confirm the statement of Miss Gilmore that oidia occur on the diploid mycelium in this species.

Some of the diplophytes exhibited "patchiness", *i.e.*, the mycelium was uneven, parts of it being white and fluffy, other parts less fluffy. Two patchy diplophytes are shown in the photographs reproduced in Plate I, Fig. 2. These mycelia when examined microscopically were revealed as including some haploid hyphae along with the diploid. The diploid mycelium No. 1 + 2 is illustrated in Text-fig 2, and it will be seen that there are entire hyphae which are haploid, at least judging from the fact that the cross-walls of these hyphae are devoid of clamp connections*. It was estimated that in such impure diplophytes not more than 10% of the hyphae are haploid.



TEXT-FIG. 2. *Hyphae at the periphery of the diploid mycelium No. 1 + 2, growing in a hanging drop of malt-extract agar. Haploid hyphae (h) are seen growing among the diploid hyphae. Drawn with the aid of the camera-lucida. Magnification 90.*

To account for the appearance of haploid hyphae among the diploid,

**Positive evidence that these clamp-connection-free hyphae are actually haploid will have to be obtained by a cytological examination. This will be undertaken by the writer in due course. It has been shown (3) that similar hyphae occur on the diploid mycelium of Collybia velutipes and that they contain but one nucleus in each cell. Hence it seems highly probable that the clamp-connection-free hyphae in Psilocybe coprophila are really haploid.*

two possible explanations occur to the writer: (i) when the two haplophytes were paired, diploidization was in some way incomplete so that certain hyphae remained haploid; or (ii) diploid hyphae gave rise to haploid hyphae by the separation of the nuclei of a dicaryon.

Buller (4, p. 248) has described the occurrence of irregularities in the appearance of the diploid mycelium of *Coprinus lagopus* and has used the word "patchy" to describe the mycelia. The patchy mycelia of Buller were derived by the pairing of a diploid mycelium with a haploid mycelium, the two being theoretically incompatible. The diploid mycelium is able to effect an imperfect diploidization of the haplophyte, probably by both nuclei of the dicaryon of the diploid mycelium passing into the haploid mycelium. This incomplete diploidization is not strictly comparable to the occurrence of patchy diplophytes in *Psilocybe coprophila*, but there may be some similarity in the two phenomena.

Had diploidization in *P. coprophila* been incomplete, it should have been possible, by making fresh transfers from the diploid mycelia and taking care to select only mycelium bearing clamp connections, to obtain "pure" diploid mycelium. This method of transfer was actually used for each of the four diploid mycelia Nos. 1 + 2, 1 + 8, 2 + 4, 3 + 4; but when the mycelia which developed on the fresh plates were examined a few days later, haploid hyphae were still present among the diploid.

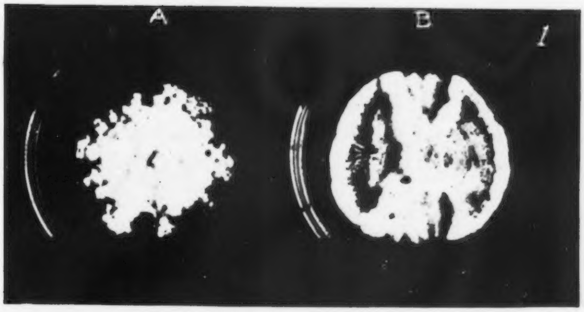
If the first hypothesis be accepted, we must assume that the hyphae which were left haploid after diploidization had taken place were altered in such a way that they were no longer capable of being diploidized by any of the other hyphae present in the culture.

When mycelium free from clamp connections was isolated from an impure diplophyte, the mycelium obtained was not haploid but again an impure diplophyte.

The second hypothesis seems more probable. As stated, it was found (3) that in *Collybia velutipes* diploid hyphae actually do give rise to haploid by the isolation of the nuclei of the dicaryon in separate hyphal branches. In cultures of *C. velutipes* it was quite easy to find the origin of the haploid branches by examining a culture of the diploid mycelium growing in a Van Tieghem cell. Bits of diploid mycelium of *Psilocybe coprophila* were planted on agar drops in Van Tieghem cells and allowed to develop. In all cultures examined in this manner, the haploid hyphae appeared to originate in the central mass of mycelium. They arose so far from the younger and less dense portions of the mycelia that it was quite impossible to form any clear idea as to how they had originated. However, it is quite possible that their mode of formation is the same as in *Collybia velutipes*.

The haploid hyphae occurring on the diplophytes of *C. velutipes* bear oidia. Thinking that a similar situation might obtain in *Psilocybe coprophila*, a very careful search through all the diploid mycelia was again made with the result that no oidia were found. It is not improbable, however, that the

PLATE I



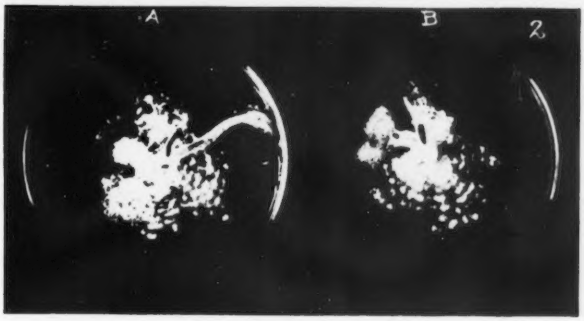


FIG. 1. *Two haploid mycelia of Psilocybe coprophila two weeks old; A, No. 1, B, No. 2. One-half natural size.*

FIG. 2. *Two patchy diplophytes: A, No. 1 + 2, B, No. 1 + 8. In A is shown an imperfect carpophore. One-half natural size.*

residual haploid hyphae occurring along the diploid hyphae might, under certain circumstances, produce oidia. Unless the mycelia were examined exceedingly carefully, the observer might easily be led to believe that the oidia were borne on diploid mycelium properly so-called.

On Plate 33, Fig. 9 of her article, Miss Gilmore illustrates oidia borne on a mycelium bearing clamp connections. The writer has failed to find anything resembling this in his cultures. There is, nevertheless, no reason for not supposing that the oidia in question might have developed on *haploid* mycelium which *later* became diploidized and bore clamp connections.

On many of the diplophytes there were produced fruit bodies, some of which developed normally, others of which were abnormal (Plate I, Fig. 2) as has been reported by Miss Gilmore.

Acknowledgment

The writer wishes to thank Dr. A. H. Smith for providing him with the carpophores of *Psilocybe coprophila** and for identifying them.

References

1. Brodie, H. J. Ann. Bot. 45 : 315-344. 1931.
2. Brodie, H. J. Ann. Bot. 46 : 727-732. 1932.
3. Brodie, H. J. The occurrence and function of the oidia of the hymenomycetes. Manuscript to be published in Am. J. Botany. 1935.
4. Buller, A. H. R. Researches on fungi, IV. 1931.
5. Gilmore, Kathryn A. Bot. Gaz. 81 : 419-433. 1926.
6. Lohman, Marion L. Papers Mich. Acad. Sci. Arts and Letters, 13 : 141-157. 1931.
7. Vandendries, René and Brodie, Harold J. La Cellule, 42 : 165-209. 1933.

* *Cultures Nos. 1, 2, 3, 4 and 1 + 2 have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland.*

ANEMOUSITE IN ESSEXITE[1]

By F. Fitz Osborne[2]

Abstract

Anemousite has been found in some Monteregian essexites. This raises some questions regarding the distribution of anemousite and the nomenclature of essexite. The name essexite may well be retained despite the occurrence of anemousite in the rock.

Introduction

The undersaturated feldspar, anemousite, of the Linosa basalts was described by Washington and Wright (10) in 1910. Nevertheless, undersaturated feldspars have not been described from many localities: Bancroft and Howard (3, pp. 22-24) have given an analysis of such feldspar from Mount Royal, and Barth (4, 5) has given the name pacificite to anemousite basalt.

The author has recently studied some of the Monteregian essexites, particularly those of Mount Johnson, and has found that undersaturated feldspar is abundant in some of them, and in some, associated with nepheline.

Anemousite from Mount Royal

There are only two trustworthy analyses of feldspar separated from Monteregian rocks and one of these, by M. F. Connor, formerly analyst for the Geological Survey of Canada, shows the presence of an undersaturated molecule in the feldspar. The analysis was published by Bancroft and Howard (3). The essexite specimen from which the feldspar was separated is from station 204+85 of the railway tunnel passing through Mount Royal. The locality is about 1000 ft. north of the south contact of the main mass of essexite with the Trenton limestone and is about 0.5 mile north of the campus of McGill University.

The rock is a rather abnormal facies of the essexite in which a well marked ophitic texture is developed; olivine is included poikilitically in augite which is rimmed with alkaline amphibole, and both augite and amphibole are moulded on laths of plagioclase. A mode for the rock has been given (3), but it does not agree with the norm of the analyzed specimen in that the mode gives too high a proportion of feldspar. The analysis of the rock by J. B. Robertson is shown in Table I.

TABLE I

Analysis of essexite from Mount Royal

SiO_2	41.55%
TiO_2	3.92
Al_2O_3	14.84
Fe_2O_3	6.62
FeO	8.24
MgO	7.83
CaO	14.64
Na_2O	1.93
K_2O	0.25
H_2O	0.19
MnO	0.15
P_2O_5	0.10
CO_2	0.19
Cl	Trace
Cr_2O_3	None
SO_3	None
S	0.16
SrO	None
BaO	None
NiO	Trace
CoO	Trace
Total	100.61
Less O for S.	0.05
	100.56

[1] *Manuscript received February 5, 1935.*
Contribution from the Department of Geology, McGill University, Montreal, Quebec, Canada.
[2] *Assistant Professor of Geology, McGill University.*

Bancroft and Howard recognized only one feldspar in this rock, but the writer has been able to distinguish two in their thin sections. One is a normal plagioclase zoned from about An60 to An82 in the core. In one thin section there is less of this plagioclase than anemousite, but in another there is more. The two feldspars differ in several respects. The plagioclase tends to be idiomorphic and the anemousite to border it, but the latter is itself somewhat idiomorphic toward amphibole and pyroxene. The indices of the more calcic zones of the plagioclase are noticeably higher than those of the anemousite. The plagioclase is twinned after the albite and Carlsbad laws with very subordinate pericline and Baveno twinning. The anemousite shows some albite twinning, but the prominent twinning is according to another law: this appears to be pericline with a composition face near 100. Sections of anemousite simultaneously perpendicular to 010 and 001 are nearly parallel to the composition face of the twins, and show the positive acute bisectrix emerging at about 20° from the centre of the field. Corresponding sections of the plagioclase show eccentric emergence of the bisectrix α. Boundaries of lamellas of polysynthetic twinning in the anemousite lack sharpness, and composition faces of the twins are irregular. The plagioclase lamellas in all sections except those much inclined to the composition face are sharply defined and straight.

The optic-axis interference figures of the anemousite show isogyres colored red on the concave side and the dispersion is thus $\rho<v$, whereas the corresponding isogyres of plagioclase are black. The dispersion is so strong that extinction is incomplete in white light, and the section changes from blue to brown near the extinction position. The anemousite is positive and the optic-axial-angle is about 60°. The indices of refraction determined on an uncovered thin section using freshly standardized oils are $\alpha=1.559$, $\beta=1.562$, $\gamma=1.566$.

Connor's analysis of the undersaturated feldspar and the molecular proportions after deductions for iron ore minerals, diopside and olivine are shown in Table II.

TABLE II

ANALYSIS OF UNDERSATURATED FELDSPAR

	Connor's analysis	Molecular proportions after deductions
SiO_2	49.06	.8086
TiO_2	0.14	
Al_2O_3	30.96	.3049
Fe_2O_3	0.55	
MgO	0.15	
CaO	13.05	.2292
Na_2O	4.79	.0773
K_2O	0.50	.0053
MnO	0.04	
Loss on ignition	0.76	

The recalculated analysis is, by weight, Or 3.00, Ab 21.81, An 64.72, Ne 10.48%, but, as shown before, the feldspar is probably a mixture of about 50% of anemousite and 50% of calcic plagioclase. If the composition of the plagioclase be estimated as 75 weight per cent anorthite, the composition of the anemousite is Or 6.00, Ab 18.62, An 54.44, Ne 20.96%. The assumption on which the recalculation is based may be in error in several particulars. The plagioclase itself may be slightly undersaturated and it may

contain some potash. However, it appears to be definitely earlier than the anemousite and is of normal optical character. In any case the analysis proves the presence of an undersaturated feldspar.

Bancroft and Howard give a specific gravity of 2.705 for the mixture. If the specific gravity of the anemousite is calculated assuming 50% plagioclase of specific gravity 2.72, the result is 2.68 which is in good agreement with the feldspar from Linosa.

Mount Johnson

If the mass of plutonic melilite rock cutting the pre-Cambrian near Oka be excluded, Mount Johnson is the smallest of the Monteregian hills in outcrop area. Nevertheless, it is one of the most interesting, for five facies of plutonic rocks arranged as vertical, hollow, coaxial cylinders may be recognized. The core is of olivine essexite, surrounded by a fine-grained essexite known in the monument-stone trade as Ebony. The next facies is also an essexite which, on account of the pellucid character of its feldspars, resembles the stone from Quincy and is known commercially as Canadian Quincy. These are surrounded by a porphyritic rock for which N. L. Wilson and the writer have coined the term monnoirite. The outer annulus is a pulaskite porphyry grading to a pulaskite near the hornfels collar that surrounds the mountain. The rock of the core is the finest grained but the granularity does not increase uniformly outward. The coarsest appears near the contact of monnoirite and pulaskite porphyry. The feldspar of the several facies at Mount Johnson shows considerable differences in composition. It was the anomalous optical properties of the anemousite in the olivine essexite that led to the critical examination of the composition of the feldspar. The anemousite is more abundant in the core of the mountain than in the coarser facies, but it is found also in the Ebony and the Canadian Quincy. The monnoirite and the pulaskite are almost saturated rocks, and the feldspar they contain appears to be of normal composition. An analysis of the feldspar from the pulaskite shows no deficiency of silica, and the feldspar of the monnoirite is of the rhomben porphyry type.

Olivine essexite from the core of the mountain is millimetre grained, with platy phenocrysts of plagioclase as much as 12 mm. long. Augite occurs in two generations: the larger crystals are as much as 9 mm. long and 2.75 mm. wide, but the smaller ones are nearly equidimensional and are 0.12–0.42 mm. in diameter. The olivine is 0.1–0.2 mm. in diameter and the opaque minerals, which form 8% of the rock, are less than 0.25 mm. in diameter. Most of the feldspar and nepheline is from 0.5 to 2 mm. in diameter. The larger crystals of pyroxene and the phenocrysts of plagioclase partake of the vertical fluidal arrangement of the constituents which is a conspicuous feature of most of the plutonic facies on the mountain.

Pyroxene is of two kinds separable in favorable cases by optical properties. Part of it is a normal titaniferous augite showing strong dispersion and having an optic-axial-angle near 60°. The other is recognizable among the

smaller grains of the first generation of pyroxene. It has no appreciable dispersion and lacks the hour-glass structure seen in the augite. The optic-axial-angle determined by Mallard's method with a microscope, calibrated by means of oriented plates of minerals of known angle and indices, and checked with an Abbe apertometer, is between 50 and 52°. This pyroxene may be rimmed by the alkaline hornblende, but such is not the case for the normal augite. The alkaline hornblende and biotite are in an ophitic relation to the feldspar and are apparently of late development. The anemousite rock from Mount Royal is markedly ophitic. Nepheline appears to have crystallized only slightly later than the bulk of the anemousite.

Apatite occurs in large crystals and in fine needles cutting silicates.

The principal interest in the rock centres on the feldspar. Adams (1, p. 258), in describing the petrography of Mount Johnson, mentions the occurrence of pyroxene, biotite, amphibole, plagioclase, potash feldspar, nepheline and accessories. Examination of Adams' thin sections fails to show any potash feldspar. The thin sections were cut about 30 years ago, and the balsam has the relatively high index of 1.545, but careful search of the thin sections failed to disclose any abundant mineral with indices less than that of the Canada balsam, proving the absence of potash feldspar.

The plagioclase phenocrysts show sharply defined albite lamellation with Carlsbad twinning and rarely Baveno and pericline twinning, the latter with the normal composition face. The cores of the crystals are as calcic as An40, but the outer parts are considerably more sodic than this, appearing on some crystals to grade outward to An20. In places anemousite appears to rim plagioclase as if it were in reaction relation to it, but most of the anemousite is alone or in a degenerate sort of coarse intergrowth with nepheline.

The anemousite is triclinic in crystallization. Albite twinning is rare and the lamellas are faint. The common twinning is apparently pericline and the composition face is near the front pinacoid, for sections showing two cleavages nearly at right angles show no twinning—presumably because they are nearly parallel to the composition face. Such faces show nearly central emergence of γ and the optic axis near the edge of the field. The optic-axial-angle, as determined by Mallard's method, is between 60 and 65°. The sign is positive. In a few places the anemousite appears to have unmixed in two feldspars with the components in lenses approximately parallel to 010. In most, if not all, orientations the junction lines of adjacent twins are blurred and far from straight. In many places they actually show re-entrant angles. The dispersion is greater than that of plagioclase but less than that of the anemousite from Mount Royal and is $\rho<v$. Of all the properties, the irregularity of the twinning lamellation and the optic-axial-angle show the greatest constancy for the anemousite, for a variation in composition is indicated by range of extinction angle (on 010, 10–30°), indices and density.

An unsuccessful attempt was made to separate the anemousite from the plagioclase and other rock-forming minerals by the use of heavy liquids. The variation in the properties came to light in the course of this work. It was possible to separate the more calcic plagioclase from anemousite, but the other fractions all showed some of this mineral, and the anemousite was distributed through several fractions. Part of the anemousite sank immediately in a solution of density 2.672, another fraction sank in four hours, yet another remained suspended in the liquid for 20 hr., and still another floated on it. In general, the lighter fractions have the lower indices of refraction. The indices for one composition ($d = 2.672$) determined by immersion are $\alpha = 1.559$, $\beta = 1.562$ and $\gamma = 1.566$.

Despite the fact that it was not possible to make a separation of the anemousite for analysis, it is possible to arrive at the composition of the mineral indirectly. Adams has given the results of a Rosiwal analysis of a specimen which was analyzed by Connor. Adams states that the determination was based on several thin sections and more than 500 intercepts were made. He noted that the quantity of nepheline was lower than that demanded by the norm, but he was inclined to attribute that result to the imperfections of the Rosiwal method. It is probable that the Rosiwal determination is substantially accurate and the deviations are due to the presence of undersaturated feldspars. His determination of the amount of nepheline was checked by etching and staining.

A number of analyses of minerals occurring in the Monteregian rocks are also available, so it is possible to calculate the composition of the anemousite from the Rosiwal and chemical analyses of the rock with reasonable accuracy. This method yields only the average composition of the feldspar, and it is necessary to assume in the calculation that the plagioclase is saturated and contains little or no potash. The optical properties suggest that this assumption is justifiable.

For the calculation, an amount of apatite sufficient to account for all the phosphoric acid was determined and deducted and the percentages by weight

TABLE III

CALCULATION OF THE COMPOSITION OF THE ANEMOUSITE FROM MOUNT JOHNSON

	%	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	MnO
Nepheline	6.00	2.60		2.06				0.06	0.98	0.31	
Pyroxene	13.25	6.74	.10	.56	0.19	0.55	2.12	2.97			0.02
Amphibole	1.26	.49	.06	.15	.05	.15	.13	.16	.04	.02	.01
Biotite	3.97	1.31	.11	.41	.35	1.09	.03	.03	.04	.31	.11
Olivine	1.36	.52				.34	.58	.03			
Opaques	7.89		1.44		2.50	4.28					.02
Apatite	2.52							1.39			
Sum		11.66	2.71	3.18	3.09	6.41	2.86	4.64	1.06	.64	.15
Analysis		48.69	2.71	17.91	3.09	6.41	3.06	7.30	5.98	3.10	.15
Difference		37.03		14.73			.20	2.66	4.92	2.46	

were then recalculated to give the same total as the chemical analysis. The compositions of the minerals were selected as follows: nepheline, Brogger's average from ijolite; augite from Mount Royal; amphibole from Mount Johnson; biotite from Mount Royal; olivine from Mount Royal. The excess of MnO, Fe_2O_3, FeO, TiO_2 are allotted to opaques. The results of the calculation are shown in Table III.

The agreement between calculation and analysis thus obtained is very satisfactory: the small excess of MgO may be present as spinel in the opaques or as clinoenstatite in the pyroxene, in any case it is so small it may be disregarded. The silica, alumina, lime, soda and potash may be allotted to feldspar, which forms 62% of the rock. The bulk composition by weight of the feldspar is Or 14.57, Ab 26.83, An 13.22 and Ne 8.06%.

The writer estimates the amount of plagioclase as about one-third of the total feldspar, or 20% of the rock. On the average it contains about 30% by weight of anorthite. The approximate average composition of the anemousite is Or 34, Ab 30, An 17, Ne 19%, after deducting plagioclase. In this case, also, potash feldspar may be dissolved in the plagioclase, and thus the amount of Or in the above composition would be reduced. This mineral might be regarded as an anemousite-bearing potash oligoclase (7). This occurrence is of particular interest in that nepheline appears as a separate phase which crystallized simultaneously with anemousite.

Bowen and Greig (6, p. 211) have suggested that the presence of carnegieite (soda anorthite) may not be the best explanation of the undersaturation of these feldspars (see Ref. 2, pp. 326-334). It is worth noting in this connection that the analyses of the Mount Royal feldspar and the calculated feldspar show less alumina than is necessary to account for the bases.

The analyses and calculations presented in this paper appear to be sufficient evidence that an unsaturated triclinic feldspar exists in some essexites.

To sum up, the anemousite feldspars of the Monteregian province appear to have a higher dispersion than the normal plagioclase, they are optically positive and the angle between the optic axes is less than in any plagioclase. The twinning bands are less distinct than those of normal plagioclase, and a lamellar twinning approximately parallel to 100 is found. It is noteworthy that some of the potash oligoclases share some of these properties. A feldspar of the rhomben type occurring in the monnoirite at Mount Johnson has a higher dispersion than the normal plagioclase. In this respect it resembles the feldspar described by Quensel (9, p. 9) which is optically positive and has a notable dispersion. A feldspar from Mount Johnson occurring in an almost saturated pulaskite porphyry was analyzed by N. L. Wilson under the supervision of the writer. The powder was treated with concentrated hydrochloric acid in order to destroy the small amount of zeolitic minerals and sodalite. This feldspar shows a high dispersion similar to that of the Mount Johnson anemousites, but the analysis shows that the feldspar is saturated; therefore, the existence of a strong dispersion must be used with caution as a criterion for anemousite.

The compositions of anemousites are given in Table IV. Of these only one, *viz.*, the feldspar from Linosa, has been analyzed alone. The Mount Royal material was diluted with plagioclase, but inasmuch as it is definitely undersaturated it is of value in proving the existence of anemousite in a plutonic rock. The compositions given by Barth and the one from Mount Johnson are calculated from the analyses of the rocks, the presence of the anemousite type of feldspar being confirmed by optical properties. It is uncertain whether solutions of such diverse composition should be included under one name. Washington and Wright (10, p. 62) anticipated that an undersaturated series of feldspars might be found and new names given for compositions different from that of the Linosa feldspar. No easy method of division appears and, until analyses of pure material are available, anemousite may be used for the whole series.

TABLE IV

COMPOSITIONS OF ANEMOUSITES

Weight per cent

	Barth pacificite							
Or	22	32	9	8	10	28	39	18
Ab	33	9	54	40	56	29	30	40
An	12	11	12	43	31	2	3	33
Ne	33	48	24	15	3	42	28	9

	Barth phonolite		Osborne Mt. Johnson	Osborne Mt. Royal	Washington and Wright Linosa
Or	14	36	34	6	4
Ab	44	55	30	19	36
An	11	7	17	54	54
Ne	31	5	19	21	6

TABLE V

COMPOSITION OF 13 ESSEXITES AND TWO OLIVINE ESSEXITES FROM MOUNT JOHNSON

	I	II	III
SiO_2	48.98	48.69	48.41
TiO_2	1.99	2.71	2.70
Al_2O_3	17.35	17.91	18.51
Fe_2O_3	3.83	3.09	4.62
FeO	6.30	6.41	5.54
MnO	.20	.15	n.d.
MgO	3.52	3.06	3.00
CaO	7.54	7.30	7.79
Na_2O	5.54	5.95	5.66
K_2O	2.73	2.56	2.14
H_2O+	1.24	.95	.97
P_2O_5	.78	1.11	1.31
	100.00	99.97	100.75

I. Osann-Rosenbusch, average of 13 essexites.
II. Olivine essexite, N. N. Evans, analyst.
III. Olivine essexite, N. L. Wilson, analyst.

Anemousite Essexite

The finding of anemousite in the Monteregian essexites raises two questions. Is the mineral common in essexites, and does its presence make it necessary to give such a rock a new name? In answer to the first question, the mineral appears to be of fairly widespread occurrence in the Monteregian essexites and in some of the nepheline syenites. In the nepheline syenites it forms a rim around plagioclase, and is not commonly twinned. The twinning lamel-

lation in some anemousite is not noticeable and doubtless it is this fact, coupled with the considerable content of potash, that influenced some petrographers in mentioning potash feldspar as a constituent of the rock. The term essexite was proposed by Sears for the rock from Essex County, Mass., but he later decided that this rock has resulted through metasomatism of a gabbro by nepheline syenite, and he mentioned the rock from Mount Johnson as a typical example of a magmatic rock of that composition. Table V shows the average composition of 13 essexites and two analyses of olivine essexites from Mount Johnson. The analysis given in Column III has not been previously published. This would suggest that the name essexite would be retained, but Barth decided that it was advisable to give the new name pacificite to some lavas of composition of essexite because of the presence of anemousite.

Note

Since the above paper was written one by Ernst and Nieland (7) has appeared. They have analyzed some feldspar from Linosa megascopically similar to that analyzed by Washington and Wright, and found no undersaturation.

References

1. Adams, F. D. The Monteregian Hills, a Canadian petrographical province. J. Geol. 11 : 239–282. 1903.
2. Ahlers, L. Über die Dichte von Quarz, Orthoklas, Albit und Anorthit. Z. Krist. 59 : 294-334. 1924.
3. Bancroft, J. A. and Howard, W. V. The essexites of Mount Royal, Montreal, P.Q Trans. Roy Soc. Can., IV, 17 : 13-42. 1923.
4. Barth, T. F. W. Pacificite, an anemousite basalt. J. Wash. Acad. Sci. 20 : 60-68. 1930.
5. Barth, T. F. W. Mineralogical petrography of Pacific lavas. Am. J. Sci., Ser. 5, 21 : 377-405; 491-530. 1931.
6. Bowen, N. L. and Greig, F. W. The crystalline modifications of $NaAlSiO_4$. Am. J. Sci., Ser. 5, 10 : 204-212. 1925.
7. Ernst, E. and Nieland, H. Tschermak Min. Pet. Mitt., 46: 93-126. 1934.
8. Mountain, E. D. Potash-oligoclase from Mt. Erebus, Antarctic, and anorthoclase from Mt. Kenya, East Africa. Mineralog. Mag. 20 : 331-345. 1925.
9. Quensel, P. Ein Vorkommen von Rhomben-porphyren in dem prakambrischen Grundgebirge des Kebnekaisgebietes. Bull. Geol. Inst. Univ. Upsala, 16 : 1-14. 1918.
10. Washington, H. S. and Wright, F. E. A feldspar from Linosa and the existence of soda anorthite. Am. J. Sci., Ser. 4, 29 : 52-70. 1910.

VISCOSITY EFFECTS IN A CHANNEL OF SMALL EXPONENTIAL DIVERGENCE[1]

By G. N. Patterson[2]

Abstract

An experimental investigation of a flow form, deduced by Blasius from theoretical considerations, was carried out with air as the medium. A photographic method of measuring velocity distributions was adopted, and a diverging channel was designed from considerations based on the theoretical treatment and on requirements arising out of the experimental method. At a Reynolds number of 35, curves of velocity distribution were measured at various positions along the channel, and comparisons were then made with the corresponding theoretical curves. Good agreement was found over the region of the channel to which the theoretical results could be applied. A study of the experimental curves in that part of the channel to which the theoretical results could not be applied quantitatively showed further that the general flow characteristics described by Blasius are to be found in this region.

Introduction

Viscosity effects in air moving near a curved surface can be conveniently studied by observing the flow of air through diverging channels. The problem of the flow of a viscous incompressible fluid through diverging channels has been treated theoretically by Blasius (1), who obtained an approximate solution for the case of a very gradual divergence. Since the motion of air at very low velocities may be regarded as essentially that of a viscous incompressible fluid, it was considered that the flow characteristics described by Blasius could be shown to exist in slowly moving air. As described by the author in the first paper on this subject (3), the Blasius flow form is to be found in the range $25 \leq R \leq 36$ of the Reynolds number. In order to investigate more thoroughly these viscosity effects near a curved surface, the present work was undertaken to obtain a quantitative comparison between the curves of velocity distribution deduced by Blasius and those obtained by direct measurement.

Summary of Blasius' Work

Blasius considered the two-dimensional flow of a viscous incompressible fluid through a channel which diverges in a gradual symmetrical manner according to a relation of the general form

$$z = f(\epsilon x), \tag{1}$$

where (x, z) is a point on the wall of the channel, and ϵ is a small quantity. The choice of the particular form of Equation (1) was made subject to two conditions:—

(1) The first derivative of z with respect to x must be small.

(2) Each differentiation with respect to x must decrease the order of magnitude by ϵ.

[1] *Manuscript received December 24, 1934.*
Contribution from the Department of Physics, University of Toronto, Toronto, Ontario, Canada.

[2] *Holder of a bursary under the National Research Council of Canada.*

By a method of successive approximations, arising out of a treatment of the orders of magnitude of the terms in the hydrodynamical equations of motion, Blasius showed that, as a first approximation, the curve of velocity distribution along any line perpendicular to the axis of the channel was a parabola of the form

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{1 - \left(\frac{y}{z}\right)^2\right\}, \tag{2}$$

where u is the component of velocity in the direction of x at any point (x, y) in the fluid, $\bar{u}$ is the mean velocity at $x = 0$, and w is the width of the channel at $x = 0$. In order to obtain a better solution, Blasius then carried out a second approximation which introduced a correction factor, and Equation (2) became

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \frac{3}{4} R \frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\}, \tag{3}$$

where R is the Reynolds number (3, p. 778, footnote). An investigation of this equation revealed the existence of two points, symmetrically situated on each wall of the channel, at which the flow leaves the wall. Beyond each point of "break-away" is a region of reversed flow in which the particles near the walls move in the negative direction of the axis of x.

Blasius used the relation

$$\left(\frac{\partial u}{\partial y}\right)_{y=z} = 0 \tag{4}$$

as the condition for the break-away. Substituting from Equation (3) he obtained

$$R\frac{dz}{dx} = \frac{35}{2} \tag{5}$$

as the equation from which may be deduced the value of x at which the break-away occurs.

Blasius also obtained an equation for the component of transverse velocity, v. Since v is of the order of $\frac{dz}{dx}$, and therefore very small compared with the axial velocity, u, it does not play an important part in an investigation of the motion of the fluid.

Method of Experimental Investigation

It was decided to investigate the photographic method of Nisi and Porter (2), which could be used for the measurement of low velocities. The method is similar to that used in ultramicroscopy. The smoke generated by burning magnesium ribbon is mixed with the air, and the magnesia particles, which are illuminated by a horizontal sheet of light, are observed on the focusing screen of a camera fitted with a microscope objective. By replacing the screen with a photographic plate, the motions of the particles can be recorded, and curves of velocity distribution can be obtained from the lengths of the particle tracks made during a known time of exposure.

From the standpoint of the present work certain modifications of this method are required. The regions investigated by Nisi and Porter were the wakes behind bodies of various shapes where the velocity is low compared with the velocity of the undisturbed flow. However, in the case of a diverging channel, it is necessary to obtain photographs which show conditions at the same instant over an area extending from one wall to the other. Thus each photograph must include tracks which are made by particles moving in regions of both low and high velocity.

Preliminary experiments on the relation of the quality of the photograph to the speed of the particle showed that the velocity of the image of the particle on the photographic plate was a very important factor. These experiments indicated that photographs of rapidly moving particles could be obtained if the velocity with which the image crossed the photographic plate could be decreased sufficiently below the speed of the particle itself. This required a reduction, rather than a magnification, of the area to be photographed.

Further experiments were then carried out to determine the feasibility of obtaining velocity distribution curves by taking a reduced photograph of the flow and examining the negative under a microscope. After many trials it was found that the smoke particles were not sufficiently distinct to yield dependable results. The experiments showed that the success of the method depended upon the weight, size, and reflecting power of the particle used. Further investigation of different types of particles revealed that the best results could be obtained with magnesium oxide dust particles.

The details regarding the optical system, the photographic arrangement, and the method of obtaining a good mixture of air and particles are contained in the author's first paper on this subject (3).

Design of the Channel

The design of the channel depends upon the conditions specified in the theoretical problem and upon restrictions arising from the experimental method. The theoretical conditions, which the channel must satisfy, are:—

(1) The walls must be curved according to some particular form of Equation (1), subject to the conditions governing this choice.

(2) The dimensions of the channel are to be such that a good two-dimensional flow is assured.

(3) If the value of $\frac{dz}{dx}$ at $x = 0$ is made sufficiently small so that it may be neglected, then the velocity distribution at $x \neq 0$ is parabolic. The restrictions placed on the dimensions of the channel by the experimental method are:—

(1) The height of the channel is limited by the relatively short distance between the camera lens and the central horizontal plane of the channel which is necessary in order to obtain good photographs.

(2) In the narrowest part of the channel (at $x = 0$) the ratio of the height of the channel to its width must be such as to allow the camera lens to collect sufficient light to produce a good photograph.

(3) For all values of x the distance between the walls is limited by the requirement that the image of both walls must appear on the negative.

The particular form of Equation (1) which Blasius suggests is

$$z = a + be^{\epsilon x}, \tag{6}$$

where a, b, and ϵ are constants to be chosen. At $x = 0$

$$a + b = \tfrac{1}{2}w. \tag{7}$$

Further

$$\left(\frac{dz}{dx}\right)_{x=0} = \epsilon b, \tag{8}$$

and hence ϵb must be small in order that a parabolic distribution of velocity may exist at $x = 0$. If $a = 0$, Equation (6) becomes

$$z = \tfrac{1}{2}we^{\epsilon x}. \tag{9}$$

Now, by the second of the theoretical conditions given above, it is necessary to choose w as small as possible, since the height of the channel is already limited by the first of the experimental restrictions. On the other hand, w must be large enough to satisfy the second experimental restriction. Investigations showed that the best conditions were obtained by putting w equal to 0.5 cm., making the ratio of the height of the channel to its width 16.

The choice of ϵ is more difficult to make since the restrictions on it are of a more general nature. According to Equation (8) the smaller ϵ is, the more nearly does Equation (3) approximate to Equation (2) at $x = 0$. On the other hand, the third experimental restriction imposes a second limit on ϵ. Equation (3) shows that the deviation of the flow conditions from the parabolic at any position in the channel ($x > 0$) depends upon the product $R\dfrac{dz}{dx}$. For any particular line (x = constant) in the channel we may write

$$R\frac{dz}{dx} = R\epsilon z = k, \tag{10}$$

where k is a constant. Consider two channels made according to two different forms of Equation (9) obtained by selecting two values of ϵ. The flow conditions along a line in the first channel will be similar to those along a line in the second channel if the product ϵz is the same for both lines, the value of R being maintained constant. Thus, as smaller values of ϵ are considered, similar flow conditions will be found to exist at correspondingly larger values of z.

This fact was verified by experiment. Tests were made with two channels for which $\epsilon = 0.1$ and $\epsilon = 0.3$, and it was found that, for the same Reynolds number, the break-away occurred at the larger value of z in the channel for which $\epsilon = 0.1$. Therefore, in order to obtain photographs in the region of the break-away, it is necessary to choose ϵ so that z is not too large.

After making a series of experimental tests it was finally decided that $\epsilon = 0.1$ gave the best compromise between the experimental and theoretical requirements. Equation (9) therefore becomes

$$z = 0.25e^{0.1x}, \tag{11}$$

which satisfies the first theoretical condition. Calculations based on this value of ϵ show that the error involved by using Equation (2), when $x = 0$, instead of Equation (3) is about 1%, which is about the same order as the error in the measurements of the particle paths.

In order to produce a parabolic distribution at $x = 0$, the walls of the channel for $x < 0$ were made straight and parallel, with a distance w between them. The value of $\dfrac{dz}{dx}$ at $x = 0$ was so small that the two parts of the channel could be joined at $x = 0$ without introducing any discontinuity. For the construction and dimensions of the channel and the experimental arrangement for producing a steady flow, the reader is referred to the author's first paper (3).

Experimental Technique

Measurements of the velocity distributions throughout the channel were carried out, at $R = 35$, where the best conditions were considered to prevail. The camera and the optical system were mounted on the same base, so that they could be moved to different points along the channel without disturbing the relative positions of the camera and the horizontal sheet of light. The steadiness of the flow was tested by taking a series of photographs at different times in the region about the line $x = 0$. Curves of velocity distribution along the line $x = 0$ were obtained from measurements of the photographs, and the mean velocity for each curve was calculated. The maximum variation was found to be less than 1%. Photographs of the particles were then taken at various distances along the channel ($x > 0$), the mean velocity being kept constant at 10.5 cm. per sec. Lines corresponding to values of x were chosen by inspection of the photographs, and the velocity distribution along each was measured. The values of $R\dfrac{dz}{dx}$ for these lines were calculated and found to be 1.46, 2.44, 3.87, 6.00 and 8.70. Beyond the value 8.70, measurements were not possible since the value of z was too great for both walls of the channel to show on the negative.

By reducing the curve for Equation (11) to the scale of the photographs and then fitting the negative to the curve, the axial line on each negative was found. The photograph could then be orientated with respect to the cross hairs of a traveling microscope. The axial line could be found very accurately by observing the positions of particles adhering to the walls. The equipment and methods used to measure the lengths of the particle tracks and the times of exposure are described in the first paper (3).

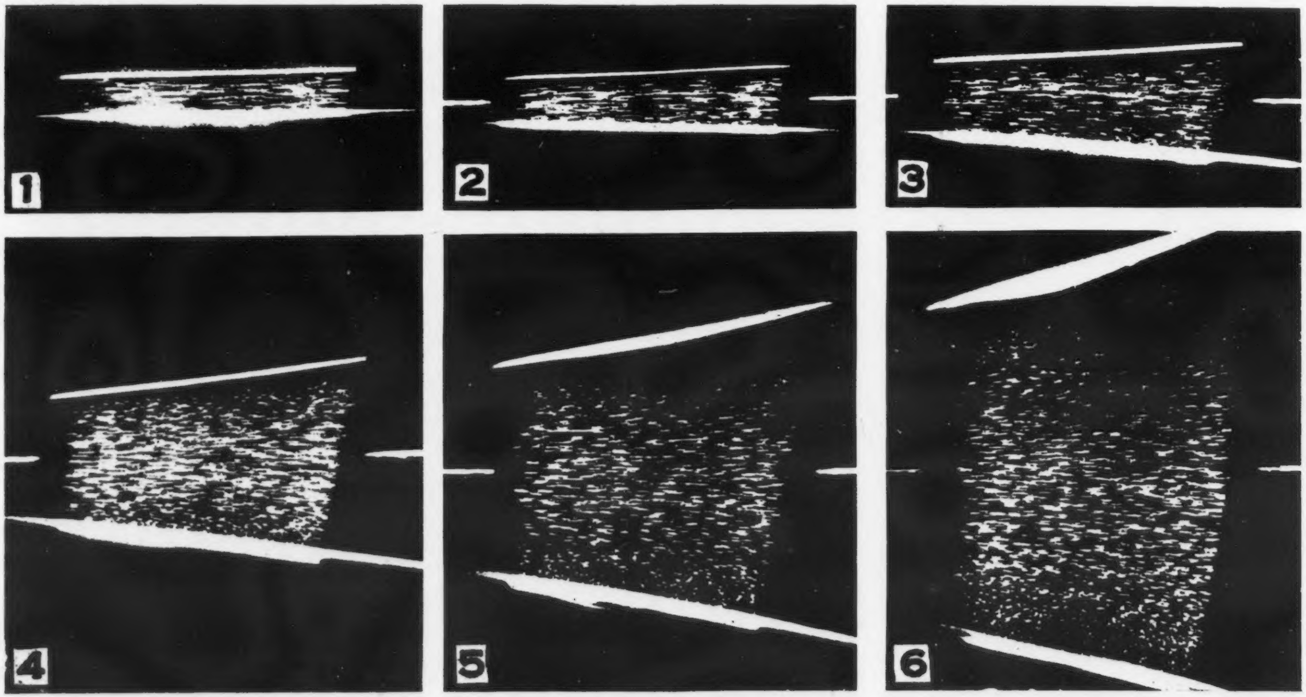


FIG. 1. *Particle tracks in the region of $x = 0$; time of exposure, 0.0166 sec.* FIGS. 2–6. *Particle tracks in regions corresponding respectively to the following values of $R\frac{dz}{dx}$: 1.46, 2.44, 3.87, 6.00 and 8.70; the times of exposure in order are 0.0166, 0.0273, 0.0393, 0.0519 and 0.0675 sec.*

Results

In Figs. 1–6 are shown enlargements obtained from the negatives from which measurements of the velocity distributions were made. Successive photographs represent the flow conditions at positions along the channel corresponding to increasing values of $R\frac{dz}{dx}$. In each case the size of the image on the negative was 0.370 times the actual dimensions. The scale of the photographs may be judged from the fact that the line joining the centre points of the two walls in Fig. 1 is the line $x = 0$ at which the width of the channel is w ($= 0.5$ cm.). The times of exposure are longer as the width of the channel increases, that is, as the velocity decreases.

Although much of the detail observed on the negative under a microscope is lost in these enlargements, they will serve to show the general characteristics of the flow. It will be seen that there is a continuous reduction of the mean velocity as the channel diverges. The gradual formation of the low-velocity region near the walls, as the divergence increases, can be traced through the photographs. Along any line drawn perpendicular to the axis of the channel, it will be seen that the velocity rises from zero at the walls to a maximum in the centre of the channel. It will be noticed that the motion is symmetrical about the axial line. Since the particle paths near the walls are very short, these photographs do not indicate the general nature of the motion in the neighborhood of the break-away. For an illustrated discussion of the motion of the particles in the reversed flow regions, the reader is referred to the first paper (3). The gradual slowing-up of the particles near the walls, which precedes the break-away, is quite noticeable in Figs. 1–6. Since the light is incident on the inside surface of one wall, some light is reflected back to the other wall. In the photographs, the line which indicates the position of the wall through which the beam passes first has superimposed on it a second longer line, which is caused by the reflection of the light. These two lines are distinguishable when the negative is viewed through a microscope, and particles which lie on the line caused by reflection can be measured. The rapid reduction of the intensity of the light with the divergence of the beam is responsible for the fact that the field on one side of the channel is brighter than on the other. This is especially noticeable in Figs. 5 and 6, where many of the very faint paths cannot be reproduced. For experimental reasons it was possible to use only the divergent part of the beam.

It was found that the components of the paths perpendicular to the axis of the channel were too small for accurate measurement. In regions of the channel where the paths were long, the inclination to the axis of the channel was small, and in regions of large inclination the paths were short. However, in order to test the symmetry of the flow, the inclinations of the paths to the cross hair of the microscope were observed. It was found that paths on the axis of the channel showed no inclinations, and that the inclinations then increased uniformly as each wall was approached.

The curves of the velocity distribution corresponding to the selected values of $R\frac{dz}{dx}$ are shown in Fig. 7. For the purpose of making a comparison, the theoretical curves given by Equation (3) are also plotted. The results show that a parabolic distribution exists at $x = 0$, and that, for $R\frac{dz}{dx} = 1.46$, theory and experiment agree, for in both cases the theoretical and experimental curves are superposed. At $R\frac{dz}{dx} = 2.44$ the theoretical and experimental results show a divergence which increases as $R\frac{dz}{dx}$ increases. The curve at $R\frac{dz}{dx} = 6$ indicates that this is the position of the break-away, and at the final position, $R\frac{dz}{dx} = 8.70$, the curve indicates the existence of a reversed flow. It should be noticed that the motion is two dimensional, since the areas under the experimental curves are equal to the areas under the corresponding theoretical curves.

Discussion of Results

In the exponential type of channel, $\frac{dz}{dx}$ increases with increasing values of x. As $\frac{dz}{dx}$ becomes larger, the approximate theoretical results, deduced on the assumption that $\frac{dz}{dx}$ is small, will become less and less accurate until values of $\frac{dz}{dx}$ are reached for which Equation (3) is no longer an approximate solution. Whether or not the theory can be expected to hold up to and beyond the points of break-away depends upon the value of $\frac{dz}{dx}$ at which the flow leaves the walls. Equation (5) shows that, unless the type of viscous flow described by Blasius occurs at Reynolds numbers of the order of 200, the condition that $\frac{dz}{dx}$ will be small up to and beyond the break-away cannot be satisfied. Thus the range in which Blasius' results may be applied depends upon the value of R. For values of R lying in the range $25 \leq R \leq 36$ it is clear that Equation (5) cannot hold, and that the approximate results of Blasius cannot be considered to hold beyond about the value

$$R\frac{dz}{dx} = 2. \tag{12}$$

The experimental results agree with this value. It can therefore be concluded that theory and experiment agree over the range of the quantity $R\frac{dz}{dx}$

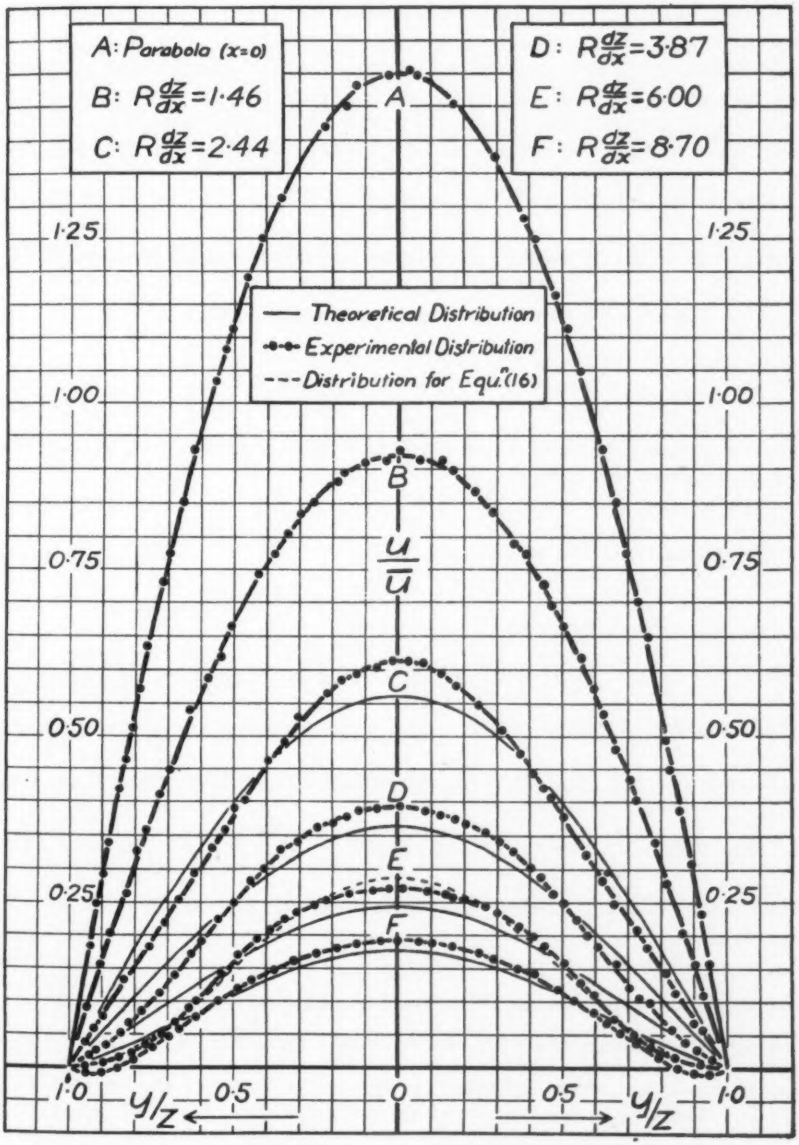


FIG. 7. *Theoretical and experimental curves of velocity distribution at various positions along the channel, showing the region of agreement between theory and experiment and the existence of a flow having the general characteristics of the type derived by Blasius.*

in which Blasius' results may be considered to hold when the Reynolds number is 35.

Although the theoretical results cannot be applied quantitatively beyond the line given by Equation (12), yet a comparison of the experimental curves in Fig. 7 with the theoretical curves shown by Blasius (1) indicates that the essential characteristics of the flow, deduced theoretically for regions of the channel in which $\frac{dz}{dx}$ is small, are also to be found in regions where $\frac{dz}{dx}$ is relatively large. It is possible, therefore, that a more general form of Equation (3) will hold in those regions for which $R\frac{dz}{dx} > 2$. If Equation (3) is written in the form

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \beta R\frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\} \quad (13)$$

where β replaces the value $\frac{3}{4}$ in Equation (3), then according to Equation (4), the break-away will occur at

$$R\frac{dz}{dx} = 6, \quad (14)$$

if the constant has the value

$$\beta = \frac{35}{16}. \quad (15)$$

Equation (13) now becomes

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \frac{35}{16} R\frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\}. \quad (16)$$

This equation is plotted in Fig. 7 and a comparison with the experimental curve for $R\frac{dz}{dx} = 6$ shows that the agreement is much better than that given by Equation (3). Hence, over the range $2 \leq R\frac{dz}{dx} \leq 6$ the value of β has risen from $\frac{3}{4}$ to $\frac{35}{16}$. Therefore β depends upon $R\frac{dz}{dx}$ and we may write

$$\beta = f\left(R\frac{dz}{dx}\right). \quad (17)$$

A further consideration of the curve for Equation (16) indicates that by adding higher powers of $\left(\frac{y}{z}\right)$ to the correction term, the shape of this curve can be made to correspond more closely to the shape of the experimental curve. These considerations lead to the conclusion that the generalized form of Equation (3) which would hold for $R\frac{dz}{dx} > 2$ is

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + F\left(R\frac{dz}{dx}\right)\Sigma\, a_n\left(\frac{y}{z}\right)^n\right\}, \quad (18)$$

where $n = 0, 2, 4, \ldots\ldots$, and the maximum value of n is greater than 6.

Acknowledgments

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References

1. BLASIUS, H. Z. Math. Phys. 58 : 225-233. 1910.
2. NISI, H. and PORTER, A. W. Phil. Mag. 46 : 754-763. 1923.
3. PATTERSON, G. N. Can. J. Research, 11 : 770-779. 1934.

MEASUREMENT OF THE VELOCITY OF SOUND IN LOW TEMPERATURE LIQUIDS AT ULTRASONIC FREQUENCIES[1]

By Arnold Pitt[2] and W. J. Jackson[3]

Abstract

An ultrasonic interferometer apparatus has been developed for the measurement of the velocity of sound in low temperature liquids. The method follows in general the conventional manner of producing sound waves in a liquid, *i.e.*, by the vibrations of a piezoelectric quartz plate driven at a high frequency. The velocity of sound in liquid oxygen and hydrogen at an ultrasonic frequency of 427 kilocycles per sec. was found to be:—oxygen, 912 metres per sec. (temp., $-182.9°$ C.); hydrogen, 1127 metres per sec. (temp., $-252.7°$ C.).

For the purpose of determining experimentally the velocity of sound in low temperature liquids, from which values of their compressibilities may be derived, a method of setting up ultrasonic vibrations in the liquid by means of a piezoelectric quartz plate was employed. Owing to the extremely low temperatures of the liquids and the relatively small quantities available, it was necessary to modify considerably the design of apparatus employed by previous workers (1,2).

Work on the velocity of sound in gases at low temperatures has been carried on at Leiden by Keesom (3,4,5), Itterbeek (3,4,5) and others. They employed an audible frequency resonance method and made accurate determinations of velocity, using a resonator chamber of fixed dimensions and varying the frequency of the sound wave to obtain resonance. By measuring the velocity of sound in dry air at 0° C. they were able to establish the accuracy of the method. Owing to the long sound wave-length relative to the size of the gas chamber resonator, correction factors were applied to take care of the effects of the wall and the end openings on the measured value of wave-length.

In selecting a method of measurement of velocity of sound in low temperature liquids, it was expected that the greatest difficulty would be met in avoiding boiling of the liquids, owing to the presence in the liquid of a metal column, and to the energy of the sound waves set up. Mechanical rigidity had therefore to be sacrificed in favor of low thermal conduction, and a resonance detector of unusual sensitivity developed so that the sound wave energy might be kept low. From work by Boyle (1) and co-workers, it was shown that at frequencies greater than 100 kc. per sec., with a liquid column of sufficient size, the relation between velocity and frequency was approximately constant.

[1] *Original manuscript received July 12, 1934.*
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Fig. 1 is a diagram of the sonic interferometer. The quartz-crystal disc and the reflector plate, R, were plane parallel with each other for all positions of the reflector. By means of the micrometer head, A, the reflector, R, could be moved up or down and readings of its position taken within 0.01 mm. Thermal loss was kept low by using very thin german silver tubing to support the mechanism in the liquid. The method of mounting the quartz plate presented the greatest difficulty of construction. Previous investigators (2, 7), working with liquids at ordinary temperatures, were able to enclose the crystal in a liquid-tight box and transmit the vibrations from the crystal to the liquid through a very thin metal diaphragm. This was accomplished by putting a drop of oil between the crystal and the diaphragm to secure intimate contact. Since this was impossible at low temperatures, a method of clamping the crystal, similar to that of Lack (6) was used. A film of platinum was sputtered over the faces of the crystal and it was mounted between the electrodes E_1 and E_2, being held very near the edges at the nodal region. This secured contact with the platinum films, the lower electrode E_2 being pressed against the crystal by means of the spring S_2. The crystal, clamp and spring were enclosed by a fibre shell F, and electrically shielded by a case H which was fastened to the main support tube G. Electrical connections to the electrodes were made by means of the tube G to which E_1 was fastened, and the lead T which ran through the shielding tube G to E_2. The whole construction was devised to slip into a Dewar flask as shown in the diagram. The dimensions of the Dewar flask were approximately 43 mm. inside diameter and 1 mm. wall thickness.

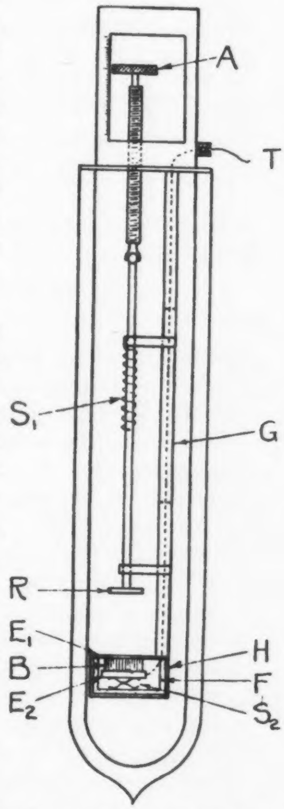

FIG. 1. *Sonic interferometer.*

The electrical wiring circuit for the ultrasonic generator is shown in Fig. 2. Section 1 of this circuit shows a crystal-controlled generator of conventional design, having a generated frequency of 427 kc. per sec. This was coupled to an intermediate screen-grid amplifier stage, which offered a high impedance path to any coupling back of energy from the output to the input stages. Section 3 shows a pentode power amplifier, having in the plate circuit a load impedance which could be adjusted by means of the condenser C_1. Radio-frequency voltage developed across this impedance was carried through C_2 and C_3 to the electrode marked E_2 in Fig. 1. E_1 was at ground potential and connected to the shielding.

To detect the condition of resonance in the interferometer system, a vacuum tube voltmeter circuit employing a sensitive detector type of tube was set up. Connection from the grid of this tube was made between C_2 and C_3. C_2 kept the d-c. potential at the plate of the power tube from being impressed

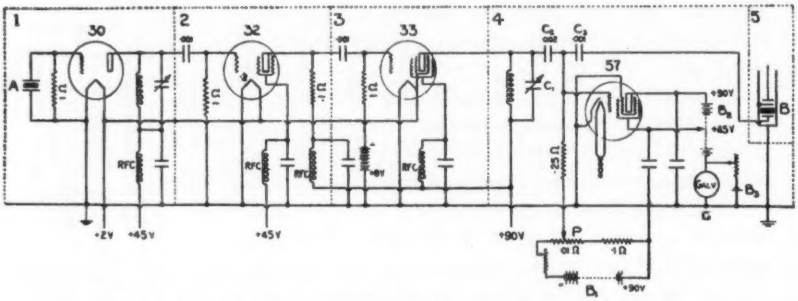

FIG. 2. *Electrical wiring circuit for the ultrasonic generator.* (*Ω = megohm; by-pass condensers are 0.1 μf.*

on the grid of the detector, and C_3 prevented the large d-c. biasing potential of B_1 from existing across the crystal and causing conduction effects in the liquids. Readings of plate-current variation were taken by means of a galvanometer having the steady value of d-c. current balanced out by means of the current from B_3. The high radio-frequency potential, which was continuously impressed at the grid of the detector, produced a large increase in plate current owing to the rectifying action of the tube. Voltage from the battery B_1 was taken from the potentiometer P to reduce the plate current and keep it at a value for which the rectifying action was a maximum.

The measurement of the wave-length of sound in a liquid in which the interferometer is immersed merely involves rotating the micrometer head A, and observing on the galvanometer in the detector circuit the periodic changes in plate current which occur as the column length between reflector and sound source is made an integral number of half wave-length distances. Vibrations in the liquid column are then in resonance with those of the crystal, with a resultant maximum amplitude in the crystal. Under this resonance condition, a sharp change in the piezoelectric voltage of the crystal occurs which may readily be detected. Knowing the half wave-length distance from the reflector displacement, and the driving frequency, the velocity in the liquid may be directly determined.

Preliminary measurements were made using liquids for which the velocity of sound is known, with a view to determining any error which might exist in the apparatus due to change in length of liquid column, effects of the walls of the Dewar flask, or other anomalous resonance effects which might be present. Owing to the exposed electrodes, only liquids of very low conductivity could be used. The sensitivity of the method was not great enough to make a determination in air at atmospheric pressure, owing largely to the method of clamping the crystal.

Having found by experiment that the piezoelectric effect was still in existence for quartz at liquid air temperatures, the interferometer was carefully immersed in liquid air and very sharp maxima were obtained. Boiling of the liquid air could not be observed for any variation in setting of the reflector, or when the oscillations of the crystal were started or stopped.

Attempts to measure the velocity of sound in liquid hydrogen, near the boiling point, were partly successful. Fairly good maxima were obtained, but some boiling was constantly going on and the presence of occluded gas in the liquid makes the result for the velocity uncertain. On manipulating the liquid hydrogen to prevent boiling, other gases were unavoidably admitted to the system. These froze and were deposited over the face of the crystal, thus preventing further measurements.

In liquid oxygen the determinations at the boiling point were very satisfactory. Exceedingly sharp maxima were obtained, and, although boiling took place at the liquid surface, conditions between the reflector and the crystal were very steady. Evaporation of the liquid oxygen to lower its temperature did not result in a sufficiently steady state for further measurements.

Table I shows results obtained for liquid oxygen and hydrogen at an ultrasonic frequency of 427 kilocycles per second. For comparison, values of velocity are given for the two elements in gaseous state and at different temperatures. All values in the table are those obtained at atmospheric pressure.

TABLE I

VELOCITY OF SOUND IN LIQUID OXYGEN AND HYDROGEN AT 427 KC. PER SEC.

—	Temp., ° C.	Velocity, metres/sec.
Oxygen		
Gas	0	315.4
Gas	−182.9	177.6
Liquid	−182.9	912
Hydrogen		
Gas	0	1286
Gas	−252.9	357
Liquid	−252.7	1127

It is intended to do further work on the measurements of these and other liquids at low temperatures. It was observed in the case of liquid hydrogen that the reaction on the detector circuit at resonance was much less than was to be expected, unless a large decrease in piezoelectric effect should have occurred. This observation was corroborated by an attempt to measure the velocity of sound in liquid helium. No reaction effects could be observed even when the temperature of the liquid was well below the boiling point.

References

1. Boyle, R. W., Froman, D. K. and Field, G. S. Can. J. Research, 6 : 102-118. 1932.
2. Hubbard, J. C. and Loomis, A. L. Phil. Mag. 5 : 1177-1190. 1928.
3. Itterbeek, A. van and Keesom, W. H. Communication from Phys. Lab. Univ. Leiden. No. 209c. 1930. No. 216c; Proc. Acad. Sci. Amsterdam, 34 : 988-995. 1931.
4. Keesom, W. H. and Itterbeek, A. van. Communication from Phys. Lab. Univ. Leiden. 209a and 213b. Proc. Acad. Sci. Amsterdam, 33 : 440-446. 1930 and 34 : 204-209. 1931.
5. Keesom, W. H., Itterbeek, A. van and Lammeren, J. A. van. Communication from Phys. Lab. Univ. Leiden. No. 216d. Proc. Acad. Sci. Amsterdam, 34 : 996-1005. 1931.
6. Lack, F. R. Bell Lab. Record, 11 : 200-204. 1933.
7. Randall, C. R. Bur. Standards J. Research, 8 : 79-99. 1932.

INTERFEROMETER MEASUREMENTS OF THE HYPERFINE STRUCTURE OF SOME LINES OF SINGLY IONIZED BISMUTH[1]

By STANLEY SMITH[2] AND J. S. BEGGS[3]

Abstract

The hyperfine structure of nine lines of Bi II and one line of Bi III has been measured by means of a quartz Lummer plate and a glass Lummer plate used in conjunction with a Hilger E 1 spectrograph. The light source was a water-cooled hollow cathode discharge in helium. Of the lines investigated the hyperfine structure of some had already been resolved either totally or in part by Fisher and Goudsmit using a 21 ft. grating, but for the lines λ6808, 6600, 4272 and 4259 the hyperfine structure has been obtained for the first time. The separation factors for the terms $6p_{\frac{1}{2}}5f_{3\frac{1}{2}}14_4$ and $6p_{\frac{1}{2}}6d_{2\frac{1}{2}}8^{\circ}_3$ have been found.

The interaction constants of the $5f_{2\frac{1}{2}}$, $5f_{3\frac{1}{2}}$, and $6d_{2\frac{1}{2}}$ electrons have been calculated.

Introduction

The most important of the terms in the spectrum of singly ionized bismuth were discovered by McLennan, McLay and Crawford (6) who at the same time found a few of the terms to have large hyperfine structure. Fisher and Goudsmit (3), by means of a 21 ft. concave grating, have investigated the structures of a number of the terms. In a later paper by Crawford and McLay (1) the analysis of this spectrum was extended. In the present investigation, Lummer plate interferometers have been used to obtain the structure of some of the lines hitherto unresolved. In the investigation of the two lines λ6600 and 6808 it was necessary to use the Lummer plate because of the difficulty of getting sufficient intensity in higher orders in this region with a grating. Other lines not previously resolved but showing structure in the interferometer patterns which have also been studied are λ4259 and 4272. The lines λ5719, 5270, 5209, 5144 and 4392 and the line λ4561 of Bi III have been re-examined.

Experimental Procedure

The source was a water-cooled hollow-cathode discharge in helium at a pressure of about 4 mm. of mercury. The Pyrex glass tube, of length 30 cm. and internal diameter 4.6 cm., had an aluminium anode and a molybdenum cathode of length 7 cm. and diameter 1.7 cm. The discharge was excited by applying a potential of about 900 volts supplied by a d-c. dynamo, and the current through the tube was normally about 0.25 amp. The light from the cathode was concentrated on the reflecting prism of the Lummer plate by means of a quartz lens, and the fringes formed were focused on the slit of a Hilger E 1 spectrograph by a quartz-fluorite lens of focal length 23 cm. The

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plane of the Lummer plate was horizontal, *i.e.*, perpendicular to the slit o the spectrograph. In order to control the position of the source relative to the spectrograph and Lummer plate, the discharge tube and accessories, such as circulating pumps, purifying tubes and helium reservoirs, formed a complete unit rigidly attached to the top of a table provided with short legs of adjustable height which rested on another table. The latter traveled on wheels along a track permitting the maintenance of the alignment of the tube with the optical system. The Lummer plate and its adjustable holder (and the Nicol prism in the case of the quartz plate) were housed in a double-walled box made of "Ten-Test". In this were windows which were formed by the quartz condensing lens and the quartz-fluorite projecting lens, the latter being rigidly attached to the spectrograph. The interferometer system and the spectrograph stood on a rigid steel base plate. As some of the exposures, especially when the red region was photographed, were of 14 hr. it was necessary to take precautions to maintain the temperature of the spectrograph and the interferometer as constant as possible. For this purpose two mercury-contact thermostats were used. One of these kept the room temperature constant with a maximum variation of about 0.5° C. and the other maintained the temperature of the box constant, the amplitude of the variation of the air temperature in the box being about 0.1° C. The temperature changes in the Lummer plate itself would probably be less than this. A glass Lummer plate, of length 13 cm. and thickness 0.4872 cm., and a quartz plate, of length 13 cm. and thickness 0.4493 cm., were used. The optic axis of the quartz plate was parallel to the long edge of the plate. When this plate was used a Nicol prism with its short diagonal horizontal was inserted between the condensing lens and the plate so that the light traveled through the plate as ordinary rays. The Lummer plate was always adjusted to give the fringes from both the top and the bottom of the plate simultaneously. The adjustment of the angle of incidence of the light on the reflecting prism of the Lummer plate to obtain symmetry between the two sets of fringes was found to be very critical. For the red region of the spectrum Eastman Process Panchromatic and Spectroscopic S III plates were used, and for the green and violet regions Eastman 33 and Process plates were used. The fringes were measured on a comparator.

Method of Measurement

As is well known in the theory of the Lummer plate, a small increase in the wave number of the light shifts an interference fringe of a given order towards the centre of the pattern, *i.e.*, the angle of emergence of the light forming the interference fringe is decreased. An important factor in the computation of the wave number differences between the components of the hyperfine structure of a spectral line is the quantity $\Delta\nu$, the change in the wave number which would make a fringe of a given order due to light of wave number $\nu + \Delta\nu$ coincide exactly with the fringe of the next lowest order

formed by the light of wave number ν. $\Delta\nu$ was calculated from the equation

$$\Delta\nu = \frac{\sqrt{\mu^2 - 1}}{2t\left(\mu^2 - 1 + \lambda\mu\frac{d\mu}{d\lambda}\right)},$$

where t is the thickness of the plate in cm. and μ is the refractive index of the plate for light of wave-length λ. To determine μ and $\frac{d\mu}{d\lambda}$ a Cauchy formula was used in the case of the glass plate. For the quartz plate the dispersion formula given in Drude's Theory of Optics (2, p. 391) was used.

Having found the value of $\Delta\nu$ for a given complex spectral line, the wave number differences between the components were computed by the method already used by McLennan and McLeod (5).

If D_1, D_2, D_3, etc., represent distances between the corresponding fringes in the two halves of the pattern due to light of wave number ν, and D_1', D_2', ... represent the corresponding quantities for fringes due to light of wave number $\nu + d\nu$, then $d\nu$ is obtained from the equation

$$d\nu = \frac{P}{Q}\Delta\nu,$$

where P is the average of $D_1^2 - D_1'^2$, $D_2^2 - D_2'^2$, etc., and Q is the average of $D_2^2 - D_1^2$, $D_3^2 - D_2^2$, etc.

This method of measurement was found to give very consistent results and is more accurate than the usually adopted method of interpolation with the set of fringes from one side of the plate only.

Experimental Results

In the following, the term nomenclature and the classification of lines are those given by Crawford and McLay (1).

λ5144. Classification $1_0^{\circ} - 8_1$

The hyperfine structure of this line has already been measured by Fisher and Goudsmit. The $d\nu$'s between the three hyperfine structure levels of 8_1, as measured by the Lummer plates, are given in Table I together with the measures of Fisher and Goudsmit.

TABLE I

OBSERVED STRUCTURE OF λ5144

f	Quartz $\Delta\nu = 0.9115$	Glass $\Delta\nu = 0.8725$		Mean $d\nu$	Fisher and Goudsmit
		Plate 1	Plate 2		
7/2	1.020	1.017	1.020	1.019	1.027 ± 0.007
9/2	0.561	0.563	0.563	0.562	0.564 ± 0.007
11/2	0	0	0	0	0

The ratio of the intervals 0.562 and 0.457 is 1.230, which differs by less than 1% from the value 1.222 predicted by the interval rule.

λ5719. Classification $2_1^0 - 7_0$

Table II gives the hyperfine structure intervals of the term 2_1^0.

TABLE II

OBSERVED STRUCTURE OF λ5719

f	Quartz . $\Delta\nu = 0.9195$			Glass $\Delta\nu = 0.8815$	Mean of measure with quartz plate	Fisher and Goudsmit
	Plate 1	Plate 2	Plate 3			
7/2	3.908	3.904	3.906	—	3.906	3.895 ± 0.006
9/2	2.143	2.145	2.144	*2.147	2.144	2.140 ± 0.006
11/2	0	0	0	0	0	0

**On the glass plate two components are blended.*

The ratio of the intervals 2.144 and 1.762 is 1.216. This is 0.5% lower than the predicted value 1.222.

λ6600. Classification $1_0^0 - 6_1$

The hyperfine structure of 6_1 has been obtained by Fisher and Goudsmit from the lines λ4705 and 4477. These lines are rather complex, consisting theoretically of nine and seven components respectively, of which eight and six were observed by Fisher and Goudsmit. The line λ6600 involves only hyperfine structure intervals of 6_1, and should therefore give a more direct and accurate measurement of the intervals. These are presented in Table III.

TABLE III

OBSERVED STRUCTURE OF λ6600

f	Glass $\Delta\nu = 0.8909$	Quartz $\Delta\nu = 0.9277$			Mean of measures with quartz plate	Fisher and Goudsmit	
		Plate 1	Plate 2	Plate 3		From λ4705	From λ4477
11/2		2.696	2.696	2.698	2.697	2.71 ± 0.01	2.68 ± 0.04
9/2	*1.197	1.213	1.213	1.215	1.214	—	—
7/2	0	0	0	0	0	0	0

**Two of the components are blended.*

The ratio of the intervals 1.483 and 1.214 is 1.222, in excellent agreement with the interval rule.

λ6808. Classification $2_1^0 - 6_1$

The structure of this line can be predicted with some certainty from the previously measured separations of the 2_1^0 and 6_1 terms.

In Table IV, the first column indicates how the components originate; *e.g.*, e arises from a transition from the $f = 9/2$ level of 6_1 to the $f = 11/2$ level of 2_1°. The second column gives the theoretical intensities calculated by the formulas of Hill (4).

TABLE IV

PREDICTED AND OBSERVED STRUCTURE OF λ6808

Change in f	Theoretical intensity	Designation of component	Predicted $d\nu$	Observed $d\nu$: Quartz $\Delta\nu = 0.9292$, Plate 1	Quartz Plate 2	Quartz Plate 3	Glass $\Delta\nu = 0.8927$
9/2→11/2	39	*e*	−1.483	−1.476	−1.482	−1.477	
7/2→ 9/2	39	*b*	−0.553	−0.547	−0.552	−0.548	
11/2→11/2	57	*h*	0	0	0	0	0
9/2→ 9/2	1.6	*d*	0.661	–	–	–	
7/2→ 7/2	25	*a*	1.209	1.223	1.220	1.219	
11/2→ 9/2	39	*g*	2.145	2.153	2.149	2.151	
9/2→ 7/2	39	*c*	2.423	2.424	2.421	2.424	2.427

For the quartz plate, a and g are blended but not quite coincident, but e and b are practically coincident. In the case of the glass plate, only h and c can be measured; the other lines form two unresolved groups.

λ5270. Classification $2_1^\circ - 8_1$

Using the intervals of the terms 2_1° and 8_1 given above, the expected structure of λ5270 was calculated.

The predicted separations between the seven components are given in the third column of Table V, together with the observed values.

TABLE V

PREDICTED AND OBSERVED STRUCTURE OF λ5270

Change in f	Theoretical intensity	Predicted $d\nu$	Observed $d\nu$: Glass $\Delta\nu = 0.8747$, Plate 1	Glass Plate 2	Quartz $\Delta\nu = 0.9135$	Fisher and Goudsmit
11/2→11/2	57	0	0	0	0	0
9/2→11/2	39	0.562	0.553	0.552	0.559	0.553 ± 0.006
11/2→ 9/2	39	2.144	2.152	2.153	*2.164	2.149 ± 0.004
9/2→ 9/2	1.6	2.706	—	—	—	
7/2→ 9/2	39	3.163	3.177	3.176	3.170	3.166 ± 0.004
9/2→ 7/2	39	4.468	4.474	4.468	4.471	3.995 ± 0.004
7/2→ 7/2	25	4.925	4.927	4.926	*4.906	4.926 ± 0.004

**These components overlap for the quartz plate.*

λ5209. Classification $2_1^\circ - 9_2$

Of the nine components of this hyperfine structure multiplet, eight have been observed by Fisher and Goudsmit. The remaining component has been found in the Lummer plate patterns. Fisher and Goudsmit give

$0.125 \pm 1\%$ as the separation factor of 9_2. It is found, however, that the separations in the hyperfine structure multiplet predicted on the assumption that the factor for 9_2 is 0.124 and that the interval rule is valid for the 9_2 hyperfine structure levels, do not agree very closely with the observations.

Another value for the factor, 0.121, was assumed. This improves the agreement between observation and prediction for some of the components, but accentuates the differences between the expected and observed separations for other components. It is therefore somewhat doubtful whether the interval rule can be regarded as applying to the term 9_2.

TABLE VI

PREDICTED AND OBSERVED STRUCTURE OF λ5209

Change in f	Theoretical intensity	Predicted $d\nu$. Separation factor of 9_2		Fisher and Goudsmit	Observed $d\nu$		
					Quartz $\Delta\nu = 0.9126$		Glass $\Delta\nu = 0.8736$
		0.124	0.121		Plate 1	Plate 2	
9/2→11/2	26	−1.491	−1.455	−1.477			
*11/2→11/2	*118	−0.809	−0.790	−0.783			
13/2→11/2	335	0	0	0	0	0	0
7/2→9/2	75	+0.095	+0.145	+0.181	—	—	0.193
9/2→9/2	155	0.654	0.690	0.688	0.662	0.666	0.673
11/2→9/2	170	1.336	1.355	} 1.383	1.353	1.355	1.356
5/2→7/2	144	1.430	1.482		1.428	1.424	1.428
*7/2→7/2	*117	1.858	1.908	1.884			
9/2→7/2	59	2.417	2.452	2.432	—	—	2.420

**These components are blended in the Lummer plate patterns.*

λ4259. Classification $8^{0}_{3} - 14_4$

This line would be expected to have 21 hyperfine structure components. Both the quartz and glass plate patterns show six components, of which one is very broad. The measured separations are as given in Table VII.

TABLE VII

OBSERVED STRUCTURE OF λ4259

Component	Observed intensity	Quartz $\Delta\nu = 0.8920$		Glass $\Delta\nu = 0.8559$	Assigned transition	Theoretical intensity
		Plate 1	Plate 2			
ϵ	2	−0.301	−0.308	−0.304		
κ	1	−0.139	−0.135	−0.136		
α	10	0	0	0	17/2→15/2	236
β	8	+0.133	+0.128	+0.128	15/2→13/2	152
γ	6	0.224	0.227	0.223	13/2→11/2	111
δ (broad)	3	0.343	0.355	0.346		

By using the graphical method described by Fisher and Goudsmit (3) and the intensity relations calculated by the formulas of Hill (4, p. 782) it is found that the data in Table VII lead to a value of B/A equal to 0.7. The assigned hyperfine structure quantum number transitions and the relative intensities are given in Table VII. The values of A and B can now be estimated and they are found to be 0.080 and 0.056 respectively.

λ4272. Classification $7^{\circ}_{2} - 13_{2}$.

Fisher and Goudsmit have calculated the separation factors of 7°_{2} and 13_{2} from their observations of the hyperfine structure of combinations of 7°_{2} and 13_{2} with other terms. The factors are 0.099 and 0.065 for 7°_{2} and 13_{2} respectively. Using these values the expected separations of the 15 components are as shown in Table VIII.

TABLE VIII

PREDICTED STRUCTURE OF λ4272

Transition	$d\nu$	Theoretical intensity	Designation	Transition	$d\nu$	Theoretical intensity	Designation
11/2→13/2	−0.906	22	*u*	3/2→ 5/2	+0.035	240	*a*
9/2→11/2	−0.620	61	*q*	7/2→ 7/2	0.079	292	*e*
13/2→13/2	−0.483	194	*v*	13/2→11/2	0.161	646	*s*
7/2→ 9/2	−0.367	112	*h*	5/2→ 5/2	0.197	189	*b*
11/2→11/2	−0.262	300	*r*	11/2→ 9/2	0.283	397	*p*
5/2→ 7/2	−0.149	172	*d*	9/2→ 7/2	0.371	208	*g*
9/2→ 9/2	−0.075	330	*k*	7/2→ 5/2	0.425	75	*c*
15/2→13/2	0	960	*w*				

TABLE IX

OBSERVED STRUCTURE OF λ4272

Observed intensity	Quartz $\Delta\nu = 0.8924$	Glass $\Delta\nu = 0.8562$	Identification of components
5	−0.447	−0.442	*v* and *g* blended
5	−0.206	−0.219	*r* and *d* blended
10	0	0	*w*
7	+0.151	+0.149	*s*
4 sharp	0.297	0.288	*p*

The Lummer plate patterns each gave five components, the separations of which are given in Table IX.

λ4392. Unclassified

This line, which as yet has not been classified, was found by Fisher and Goudsmit (3) to have three components. The interference patterns obtained with both the quartz and glass plates also showed this line to be a triplet.

In Table X are given the separations of the components.

TABLE X

OBSERVED STRUCTURE OF λ4392

Quartz $\Delta\nu = 0.8957$		Glass $\Delta\nu = 0.8594$		Fisher and Goudsmit
Plate 1	Plate 2	Plate 1	Plate 2	
0.598	0.602	0.601	0.607	0.64 ± 0.02
0.244	0.251	0.242	0.247	0.30 ± 0.01
0	0	0	0	0

λ4561

The hyperfine structure of this line of Bi III, classified as $7s\ ^2S_{1/2} - 7p\ ^2P^{\circ}_{1/2}$ has already been measured by Fisher and Goudsmit. The Lummer plate measurements are given in Table XI.

TABLE XI

OBSERVED STRUCTURE OF λ4561

Glass $\Delta\nu = 0.8636$		Quartz $\Delta\nu = 0.8999$	Mean $d\nu$	Fisher and Goudsmit
Plate 1	Plate 2			
2.891	2.891	2.883	2.888	2.88 ± 0.01
2.354	2.351	—	2.353	2.36 ± 0.01
0.534	0.533	0.538	0.535	0.52 ± 0.01
0	0	0	0	0

The hyperfine structure of the $7s\ ^2S_{1/2}$ and $7p\ ^2P^o_{1/2}$ terms are thus found to be 0.535 and 2.353 respectively, giving separation factors 0.107 and 0.471.

Discussion

The determination of the separation factor of the 14_4 term completes the separation data of the four terms 10_2, 12_3, 13_3 and 14_4 of the $6p_{1/2}5f$ configuration.

In Table XII the observed separation factors A are given, together with the values in terms of the factors for the individual electrons expected if the coupling were (j, j), p'' referring to the $p_{1/2}$ electron, f' and f'' referring to the $f_{3 1/2}$ and $f_{2 1/2}$ electrons respectively.

TABLE XII

SEPARATION FACTORS OF $6p_{1/2}$ $5f$ TERMS

Term		A observed	A for (j, j) coupling
$6p_{1/2}5f_{2 1/2}$	10_2	−0.008	$-a''/6 + 7f''/6$
$6p_{1/2}5f_{2 1/2}$	12_3	−0.018	$a''/6 + 5f''/6$
$6p_{1/2}5f_{3 1/2}$	13_3	+0.065	$-a''/8 + 9f'/8$
$6p_{1/2}5f_{3 1/2}$	14_4	+0.056	$a''/8 + 7f'/8$

The smallest value given by Fisher and Goudsmit for the 12_3 has been taken. An incomplete analysis of λ4340 from the Lummer plate data indicates that A for 12_3 should be approximately −0.016.

As Crawford and McLay (1) have pointed out, the data for the first three terms indicate that the coupling cannot be regarded as being strictly (j, j), so that it is only appropriate to apply the summation relations. However, the separation factor of the 14_4 term is independent of coupling, so if the well established value $a'' = 0.465$ is taken, f' is found to be −0.002.

The summation relations lead to the following equations

$$A(14_4) + A(13_3) + A(12_3) + A(10_2) = 2(f' + f'')$$

$$A(14_4) + A(13_3) + A(12_3) = \frac{a''}{6} + 2f' + \frac{5}{6}f''$$

$$A(14_4) = \frac{a''}{8} + \frac{7}{8}f' .$$

These equations, together with the data of Table XII, give two values for $f' + f''$, *viz.*, 0.048 and 0.034. These are in as close agreement as could be expected from the limits of accuracy of the data. It might be concluded that the term classification is correct and that the terms are not appreciably perturbed. If the average value for $f' + f''$ is taken, then $f'' = 0.043$.

TABLE XIII

SEPARATION FACTORS OF $6p_{1/2}$ $6d$ TERMS

Term		A observed	A for coupling
$6p_{1/2}6d_{1 1/2}$	5^o_1	+0.127	$a''/4 + 3d''/4$
$6p_{1/2}6d_{1 1/2}$	6^o_1	−0.165	$-a''/4 + 5d''/4$
$6p_{1/2}6d_{2 1/2}$	7^o_2	+0.099	$-a''/6 + 7d'/6$
$6p_{1/2}6d_{2 1/2}$	8^o_2	+0.080	$a''/6 + 5d'/6$

With the evaluation of the separation factor of the term 8^o_2 the data for the four terms arising from the configuration $6p_{1/2}$ $6d$ are completed as set forth in Table XIII.

It is clear from the data that the coupling cannot be (j,j) but the separation factor of the term 8°_{3}, being independent of coupling, leads to a value of 0.003 for d' if a'' is taken to be 0.465.

The summation relations lead to the following equations:

$$A(8^{\circ}_{3}) + A(7^{\circ}_{3}) + A(5^{\circ}_{2}) + A(6^{\circ}_{1}) = 2(d'_{2} + d'')$$

$$A(8^{\circ}_{3}) + A(7^{\circ}_{2}) + A(5^{\circ}_{3}) = \frac{a''}{4} + 2d' + \frac{3}{4}d''$$

$$A(8^{\circ}_{3}) = \frac{a''}{6} + \frac{5}{6}d'.$$

These equations give two widely different values for the sum $d' + d''$, *viz.*, 0.070 and 0.248. This discrepancy must be attributed to perturbation of the terms 5°_{2} and 7°_{2} by the term 4°_{2}, as suggested by Crawford and McLay (1).

References

1. Crawford, M. F. and McLay, A. B. Proc. Roy. Soc. (London), A 143 : 540-557. 1934.
2. Drude, P. Theory of optics (translated by Mann, C. R. and Millikan, R. A.), Longmans, Green and Co. New York. 1907.
3. Fisher, R. A. and Goudsmit, S. Phys. Rev. 37 : 1057-1068. 1931.
4. Hill, E. L. Proc. Nat. Acad. Sci. 15 : 779-784. 1929.
5. McLennan, J. C. and McLeod, A. R. Proc. Roy. Soc. (London), A 90 : 243-254. 1914.
6. McLennan, J. C., McLay, A. B. and Crawford, M. F. Proc. Roy. Soc. (London), A. 129 : 579-588. 1930.

THE MEASUREMENT OF SOME THERMAL PROPERTIES OF DEUTERIUM OXIDE, AND THEIR INTERPRETATION[1]

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Abstract

Values for the heat capacities of solid deuterium oxide and the resulting liquid, from initial temperatures between 4° C. and −78.5° C. to a final temperature of 25.0° C., have been determined. The specific heats of the solid over the temperature range 0 to −70° C. have been measured. The latent heat of fusion of deuterium oxide has been determined. The specific heat of liquid deuterium oxide is shown to be greater than that of water in the temperature region in which measurements were made. A comparison of the thermal properties of deuterium oxide with those of hydrogen oxide has been made, and certain points of interest are indicated.

Introduction

Some years ago a technique (1, 2) was developed in this laboratory for the measurement of specific heats and latent heats of fusion at moderately low temperatures. An investigation is at present under way to improve the calorimeter so as to ensure a much higher degree of accuracy and, at the same time, extend the temperature range over which precision measurements can be made. As a preliminary, the method previously employed (1, 2) was used for a redetermination of the specific heat of ice. The data previously published were confirmed to within the accuracy stated. As this method is admirably adapted for the purpose, it was considered of interest to measure the thermal properties of deuterium oxide.

Experimental

The procedure described previously was followed closely except that the deuterium oxide was distilled into the platinum container through a platinum tube gold-soldered to the filling tube. A capillary glass tube led into the platinum tube near its end and was cemented into place. The distillation was carried out *in vacuo*, and rigid precautions were taken to avoid contamination of the deuterium oxide by moisture from the air. The deuterium oxide, a 12 gm. sample, was obtained from the Ohio Chemical Company and was 98% pure. The thermostatic control of the required temperatures, the manipulation of the adiabatic radiation thermocouple calorimeter, the corrections and method of calculation have been described (1, 2) and need not be repeated here.

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Results

In Table I are shown the total heat changes of 1 gm. of deuterium oxide when warmed up from various temperatures to 25° C. Each value is the mean of three or more determinations. Thus the determinations at −10° C. gave 103.8, 103.7, 103.9. The accuracy with which the total heats were determined is thus about 0.2%.

TABLE I

HEAT CAPACITIES OF DEUTERIUM OXIDE

Initial temp., ° C.	4	3.8	2	0	−5	−10	−30	−78.5
Heat obs., cal.	21.6		96.26	98.2	101.0	103.8	113.5	134.5
Heat calcd., cal.		95.9		98.0	100.9	103.7	113.9	134.6

The last line contains the values calculated from the empirical equation

$$H = 183.22 - 0.2703\ T + 0.000654\ T^2 - 0.00000296\ T^3 + \frac{2.79 \times 10^{11}}{T^5}.$$

The object of finding such an equation was to facilitate the calculation of the specific heats by differentiation of the equation. The first value in Table I is the heat required to warm up liquid deuterium oxide from 4 to 25° C. The largest number of determinations were made at this temperature, since the heat change is smallest and the accuracy is least. The average specific heat of liquid deuterium oxide is 1.028 over this range of temperature, and this value probably is correct to one-half of one per cent.

The latent heat of fusion can be determined by graphic methods, *i.e.*, by extrapolating the heat curves for solid and liquid and measuring the distance between them at 3.8° C., or by using the above equation for the heat change of the solid and an equation, $H = (25 - t)\ 1.028$, for the heat change of the liquid. The first method gives 74.2 and the second 74.0 cal. The determination at + 2° C. was not used in the calculations because at this temperature the solid is so close to its melting point. This has been discussed in an earlier paper (5). The latent heat of fusion, therefore, may be taken as 74.2 ±0.2 cal.

TABLE II

SPECIFIC HEATS OF SOLID DEUTERIUM OXIDE

Temp., ° C.	Specific heat, cal. D_2O	Specific heat, cal. H_2O
0	0.579	0.4873
−10	0.545	0.4770
−20	0.514	0.4647
−30	0.484	0.4504
−40	0.457	0.4340
−50	0.432	0.4160
−60	0.410	0.3958
−70	0.391	0.3737

In Table II are given the specific heats of solid deuterium oxide at various temperatures and, for comparison, those of ordinary ice at the same temperatures.

Discussion

The specific heats of both liquid and solid deuterium oxide are greater than those of ordinary water; the latent heat of fusion however is less. The latter is the only thermal constant of deuterium oxide previously reported.

It was not directly determined but was calculated by La Mer and Baker (4) from the lowering of the freezing point. Their two estimated values, 79.9 and 75.4 cal., are both greater than the value obtained in the present investigation.

As mentioned previously 98% deuterium oxide was used in the present study. Extrapolation to 100% does not change appreciably the values given above. Thus, in the case of the latent heat of fusion it makes a difference of only 0.1 cal.

A comparison of the thermal constants of water and of deuterium oxide on a molecular basis is of interest. The specific heats of solid D_2O are greater than those of ice, so that the molecular heats are still greater. This is to be expected especially if, as present data (3) suggest, they have the same structure with almost the same interatomic distances. In that case the deuterium oxide with its heavier atoms should have the greater molecular heat. When the structure of solid D_2O has been definitely confirmed by X-ray analysis, it may be possible to calculate the relative frequencies of vibration from the specific heat data.

It was pointed out in a recent paper (6) that in liquid D_2O the equilibrium between associated and non-associated molecules $n(D_2O) \rightleftarrows (D_2O)n$ probably is shifted more to the right than in the case of water. With rise in temperature there are more associated $(D_2O)n$ molecules to dissociate, and consequently the molecular heat of the liquid D_2O should be greater than that of water. As shown above even the specific heat is greater.

On the basis of the above equilibrium it might be predicted that conversely the molecular heat of fusion of deuterium oxide should be less than that of ice, because it melts to form a liquid having a higher concentration of associated molecules. On the other hand, the higher temperature at which it melts and the increased mass of the molecules should increase the molecular heat of fusion of D_2O as compared with that of ice. The fact that they are nearly the same, 1484 for D_2O, 1436 for water, shows that the counteracting effects almost balance, and consequently the idea of greater apparent association in liquid D_2O is corroborated.

Acknowledgment

Acknowledgment is hereby made to the anonymous donors of a grant of $1,000 which has made possible this and subsequent calorimetric investigations, one of which will include further work on deuterium oxide.

References

1. Barnes, W. H. and Maass, O. Can. J. Research, 3 : 70-79. 1930.
2. Barnes, W. H. and Maass, O. Can. J. Research, 3 : 205-213. 1930.
3. Bernal, J. D. Proc. Roy. Soc. A 144 : 24-25. 1934.
4. La Mer, V. K. and Baker, W. N. J. Am. Chem. Soc. 56 : 2641-2643. 1934.
5. Maass, O. and Waldbauer, L. J. J. Am. Chem. Soc. 47 : 1-9. 1925.
6. Van Cleave, A. B. and Maass, O. Can. J. Research, 12 : 57-62. 1935.

HEAT CAPACITY MEASUREMENTS ON GELATIN GELS.[1] III.

By W. R. Horn[2] and J. H. Mennie[3]

Abstract

The heat required to warm a gelatin gel from 0° to 25° C. is greater than the sum of the heat capacities of the water and the gelatin present by an amount which varies with the concentration of the gel, and which equals 6.7 cal. per gram of dry gelatin for gels of concentration below about 52%. It is inferred that there is less bound water, or the water is less firmly bound, at 25° than it is at 0° C. If the heat capacity measurements are plotted against gel concentration, there is a sharp discontinuity at 0.52 gm. water per gram of dry gelatin, which is interpreted as meaning that this is the amount of water which is closely bound at 0° C.

Introduction

In the course of work previously reported (2) it was found that the heat capacity of a gelatin gel between 0° and 25° C. is greater than the sum of the heat capacities of the water and gelatin which it contains. This was attributed to the occurrence, within this temperature range, of some change, accompanied by a heat effect, in the gelatin-water relation in the gel. Furthermore, it was observed that there appeared to be a sudden increase in the heat effect between 61 and 67% gel concentration. It was thought worth while to investigate further by making a number of measurements of heat capacity between 0° and 25° C. on gels of varying concentration.

Experimental

The apparatus and method were the same as previously described (1, 2). The material also was the same, Eastman Kodak Co. ash-free gelatin, Lot No. 48. Gels were prepared as before by dipping the air-dry gelatin in water for a definite length of time, rapidly removing surface water with filter paper and pressing the moist material into the Monel metal container. At the same time duplicate samples were taken for analysis. The gel was then heated for half an hour at 50° C. in the sealed container. A few more dilute gels were prepared by heating the gelatin with the desired amount of water for half an hour at 50° C. and then pouring it into the container. The water content was determined by heating for 24 hr. at 105° C., which was found to be sufficient for the samples to come to constant weight.

The container was kept in an ice-water bath for at least one hour before it was transferred to the calorimeter. The container used was really designed for use with frozen gels where the heat effects to be measured were considerably larger. The temperature drop in the calorimeter was about 0.5° to 0.7°, of which only about one-third was caused by the gel, the remainder being due to the heat capacity of the container. The temperature drop was read on a Beckmann thermometer, and is the determining factor which

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limits the accuracy of the heat measurements. Two or more runs were usually made with each specimen of gel, and the results, which are shown in Table I, represent in most cases the mean of duplicate determinations.

TABLE I

% Gel	Heat capacity per gm. gel, cal.	Gm. water per gm. dry gelatin	Heat capacity of water per gm. dry gelatin, cal.	Gm. gelatin per gm. water	Heat capacity per gm. water, cal.
18.7	22.9	4.35	115.4	0.230	26.5
28.1	22.2	2.56	71.9	0.391	28.1
36.0	20.9	1.78	51.0	0.563	28.7
49.6	19.5	1.02	32.3	0.984	31.8
51.4	19.6	0.945	31.1	1.06	32.9
55.8	18.1	0.792	25.3	1.26	31.9
58.0	17.7	0.724	23.4	1.38	32.3
59.4	17.2	0.683	21.8	1.46	31.9
61.8	16.4	0.618	19.4	1.62	31.4
62.5	16.2	0.600	18.8	1.67	31.3
63.0	15.7	0.589	17.8	1.70	30.3
64.0	15.4	0.563	17.0	1.78	30.2
65.6	16.4	0.524	17.9	1.91	34.1
66.1	16.1	0.513	17.2	1.95	33.5
66.2	15.9	0.510	16.9	1.96	33.1
66.3	15.7	0.508	16.6	1.97	32.7
67.2	14.2	0.488	14.0	2.05	28.7
67.5	14.1	0.482	13.8	2.08	28.7
68.7	14.0	0.455	13.3	2.20	29.2
71.2	13.0	0.404	11.1	2.47	27.4

In Column 2 of Table I is given the total heat capacity per gram of gel between 0° and 25° C., obtained directly from the calorimetric measurements. Multiplying these figures by the weight of gel which contains 1 gm. of dry gelatin and subtracting the heat capacity of the dry gelatin (2) gives the apparent heat capacity of the water associated with 1 gm. of dry gelatin (Column 4). This is represented graphically in Fig. 1. The upper curve covers the range up to 8 gm. of water per gram of gelatin; the lower curve shows on a larger scale the region of the more concentrated gels, up to 2 gm. of water per gram of gelatin. The points previously obtained (2) are shown by the solid black circles. For gels containing more than 1 gm. of water per gram of

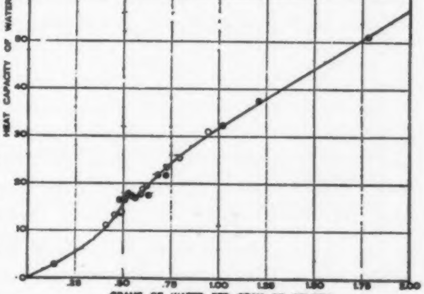

FIG. 1. *Heat capacity of water associated with one gram of dry gelatin in gels of various concentrations.*

gelatin these agree perfectly with the present results. For the three points at 0.72, 0.63 and 0.48 gm. of water, the agreement is less satisfactory although the discontinuity in the neighborhood of 0.5 gm. water per gram of gelatin, which was indicated by these earlier measurements, is now clearly confirmed.

The graph shows the change in heat capacity as increasing amounts of water are added to one gram of dry gelatin. The slope of the curve at any point then indicates the heat capacity of a small increment of the water content of the gel at that concentration. As might be expected, the initial slope is small, corresponding to a specific heat of less than 1 for the first portion of the water absorbed by the gelatin. The slope rapidly increases beyond that corresponding to the heat capacity of ordinary free water. The "apparent heat capacity" of course includes the actual heat capacity of the water and also the heat effect mentioned in the opening paragraph. This heat effect evidently appears in quite concentrated gels, and increases rapidly to a maximum at 0.52 gm. of water per gram of gelatin. It drops then suddenly as far as 0.57 gm. of water, then begins to rise again more gradually. Beyond about 0.9 gm. of water (52% gel) the curve becomes a straight line with slope corresponding exactly to the heat capacity of ordinary free water. At any point on this straight-line portion of the curve, the total heat capacity of the water in the gel is 6.7 cal. greater than that of an equal weight of ordinary free water. That is, the observed heat effect per gram of gelatin has a constant value of 6.7 cal. in gels of less than about 52% concentration.

Evidently, as far as its heat capacity is concerned at least, it is only the water in excess of about 0.9 gm. per gram of dry gelatin which is not influenced by the presence of the gelatin and may be described as "free" water. This is made further apparent by arranging the data in a slightly different manner. Dividing the figures in Column 4 by the weight of water associated with 1 gm. of dry gelatin (Column 3) gives the apparent heat capacity per gram of the water in the gels (Column 6). This is plotted in Fig. 2 against the weight of gelatin per gram of water. As increasing amounts of gelatin are added to a constant quantity of water, the heat effect observed in the gel increases in direct proportion to the amount of gelatin present, up to about 1.1 gm. of gelatin per gram of water. Beyond this point it begins to decrease. Evidently at this concentration (52%) all the water is more or less bound by the gelatin. As the ratio of gelatin to water is further increased, the amount of water held by the gelatin is now less than the maximum and the heat effect, which must arise from some change in the relation between gelatin and "bound" water, also falls off from the maximum value.

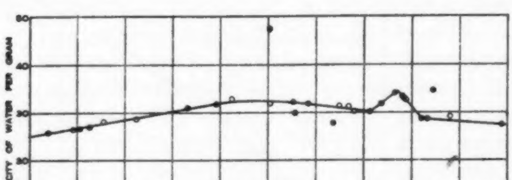

FIG. 2. *Heat capacity per gram of the water in gels of various concentrations.*

Discussion of Results

The precise nature of the changes occurring in the gel is not completely obvious. It may readily be imagined that a rise in temperature might weaken the forces of attachment between water and gelatin; that a portion of the water might be released as the temperature is raised; and that energy would have to be supplied in excess of the amount required merely to effect the increase in temperature. It would follow that in a gel at 25° C. there is less bound water, or the water is less firmly bound than at 0° C. Apparently about 0.9 gm. of water per gram of gelatin is held at 0° C. When the temperature is raised to 25° C. a portion of this is set free with the absorption of 6.7 cal. of heat per gram of dry gelatin. When the gel concentration is greater than this, the amount of water held by the gelatin is less, whence naturally the amount liberated between 0° and 25° C. and the corresponding heat required are also less.

On this basis, to account for the peculiar break in the curve between 0.52 and 0.57 gm. of water per gram of gelatin, it seems necessary to assume the existence of two distinct states or types or degrees of binding, and further, to postulate that the more firmly held portion of the water, which is obviously closest to the gelatin, either cannot escape into the less firmly bound region or, more probably, can do so only to a definite and constant extent, as long as a certain minimum amount of water is present in that outer layer. This minimum is then reached at 0.57 gm. of water per gram of gelatin and the heat effect is also a minimum at this point. When the amount of water is a little less than this, the outer layer of loosely bound water is incomplete at 0° C. and an increased amount of the more firmly bound water escapes as the temperature is raised, with an increase in the observed heat effect. At 0.52 gm. of water per gram of gelatin the outer layer has disappeared entirely and all the water is in the firmly bound state at 0° C. The amount which is released as the temperature is raised is then a maximum. Further increase in gel concentration means a decrease in the amount of water present and a decrease in the heat effect.

The extent of the decrease in the heat effect which occurs between 0.9 and 0.57 gm. of water per gram of gelatin seems surprisingly large if the water in this region is only loosely bound to the gelatin. Moreover Rosenbohm's (4) measurements on the heat of swelling, which were made with a Bunsen ice calorimeter at 0° C., indicated that the liberation of heat is practically complete when about 0.5 gm. of water has been absorbed per gram of gelatin. The results of the present work indicate that the heat of swelling should be less at 25° than at 0° C., the difference amounting to 6.7 cal. when an amount of water greater than 0.9 gm. is added to 1 gm. of dry gelatin. Some measurements of the heat of swelling are now in progress.

Since this work was started, some very interesting results along the same lines have been published (3). The heat of swelling of gelatin has been measured at 18° and at 50° C. A difference in the integral heat of swelling at these temperatures appears for amounts of water more than 0.18 gm. of water

per gram of gelatin. The difference reaches a maximum of about 12 cal. when about 0.6 gm. of water has been added. The authors infer the existence of a heat effect which they call "heat of gelatinization." They attribute it to changes taking place within the gel, of which the sol-gel transformation, which occurs within the temperature range of their investigation, is an outward and visible manifestation. They consider an explanation in terms of alteration in the gelatin-water relation, along the lines suggested in this paper, and dismiss it as being of, at most, secondary significance, on the ground that the heat effect observed is greater than is to be expected on the basis of changes in the "water-mantle" of the gelatin. They regard the principal source of the heat effect as being more probably a transformation from one modification of gelatin to another as the temperature is raised. They quote evidence from optical rotation and X-ray measurements which suggests the existence of such a transformation.

They conclude from their results that the temperature at which this transformation begins depends on the water content of the gel. At 0.230 gm. of water per gram of gelatin it begins only at 30° C.; at 0.6 gm. of water per gram of gelatin it begins at 15° and is complete at 40° C. This appears to be in general agreement with the present work, although their curves show no sign of the discontinuity between 0.52 and 0.57 gm. of water, which is so striking a feature of the results reported here.

References

1. Hampton, W. F. and Mennie, J. H. Can. J. Research, 7 : 187-197. 1932.
2. Hampton, W. F. and Mennie, J. H. Can. J. Research, 10 : 452-462. 1934.
3. Holleman, L. W. J., Bungenberg de Jong, H. G. and Tjaden Modderman, R. S. Kolloid-Beihefte, 40 : 211-240. 1934.
4. Rosenbohm, E. Kolloid-Beihefte, 6 : 177-200. 1914.

THE CATALYTIC DEHYDRATION OF ETHYL ALCOHOL BY ALUMINA

I. THE EFFECT OF THE WATER CONTENT OF THE CATALYST[1]

By L. A. Munro[2] and W. R. Horn[3]

Abstract

The dehydration of ethyl alcohol has been studied at 250° C. using alumina catalysts differing in water content. There is an optimum water content for the greatest activity of the catalyst. The apparent poisoning is greatest for those catalysts having greatest activity. There is no apparent poisoning for a catalyst having zero water content. In no case was the course of the reaction changed.

Introduction

The decomposition of ethyl alcohol may proceed in three ways—

$$
\begin{array}{lllll}
(1) & C_2H_5OH & \longrightarrow & CH_3CHO & + H_2 \\
(2) & C_2H_5OH & \longrightarrow & C_2H_4 & + H_2O \\
(3) & 2C_2H_5OH & \longrightarrow & (C_2H_5)_2O & + H_2O.
\end{array}
$$

Adkins and Krause (1) consider that the size of the pores in an oxide catalyst will determine to a large extent the type of reaction induced; *i.e.*, whether it will be dehydrogenation, dehydration or decarboxylation in the case of an ester.

Several workers have reported that a change in structure of alumina takes place between 200 and 250° C. Accordingly, in the activation of the gel at temperatures above 250° C. a change in the specificity of the catalyst might be induced.

Engelder (2) has studied the effect of added water on the reaction. He found that the presence of water in the alcohol was unfavorable to dehydration, and when present in large amounts, induced dehydrogenation. The results of Munro and Johnson (7) on the sorption of ether by alumina, and of Munro and McCubbin (8) on the catalytic reaction of carbon disulphide and water, would lead one to expect an *optimum* water content of the alumina for catalysis.

It seemed of interest, therefore, to study the behavior of a typical dehydration catalyst when its water content and the temperature of activation are varied.

Materials

The alumina used was the "moist" type supplied by the British Drug Houses. The lumps were dried for eight hours at 110° C. and then broken up to 10 mesh. Activation was carried out by heating the catalyst tube and contents in an electric furnace for two and one-half hours, at a constant temperature determined by means of a thermocouple. During activation

[1] *Manuscript received February 26, 1935.*
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and cooling, a stream of nitrogen was passed through the catalyst. The residual water content of the sample was determined by weighing the catalyst before and after activation. The total water content was determined by blasting a sample for 40 hr. over a Meker burner. In Table I are shown the activation temperatures and residual water contents.

TABLE I

ACTIVATION TEMPERATURES AND RESIDUAL WATER CONTENTS

Activation temp., °C.	300	320	410	450	500	550	580
Residual water, %	13.8	12.7	8.5	5.5	4.3	2.3	1.4

NOTE:— *Time of heating, 2½ hr. The residual water content was reduced to 0.0% by blasting with a Meker burner for 40 hr.*

Commercial 95% ethyl alcohol was first refluxed with freshly precipitated mercuric oxide to remove traces of aldehydes. This was followed by two reflux treatments, of several hours each, with quicklime. The product obtained was well over 99% ethyl alcohol.

Experimental Method and Apparatus

The general method consisted in passing a regulated flow of the vaporized alcohol (25 cc. in 90 min.) over the catalyst kept at constant temperature, condensing the liquid reaction products and unchanged alcohol, and collecting any residual gas over saturated brine.

The apparatus is shown in Fig. 1. After trying numerous methods for the introduction of the liquid, it was found that a long burette, *L*, was very satisfactory when the top 25 cc. was used. The preheater, *H*, in the form of a spiral, was placed in a small furnace the temperature of which was maintained 10° C. higher than that of the catalyst. The latter was kept at 250 ± 1° C. by a DeKhotinsky regulator, *R*. The catalyst tube, *C*, was made of 12 mm. Pyrex tubing, 24 cm. long, each end being sealed to 4 mm. tubing. Similar amounts of catalyst were used in each run.

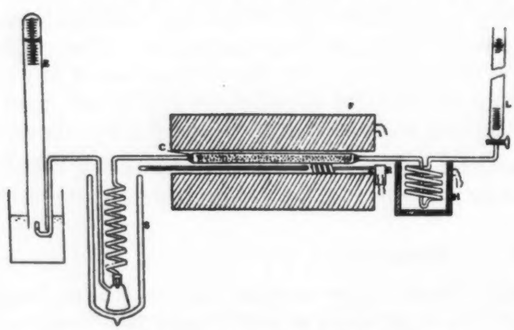

FIG. 1. *Diagram of apparatus.*

Numerous investigators have used a salt freezing mixture for the condensation of the liquid products. With this method the loss of some of the ether from the receiver is probable, especially if gas is evolved. The use of solid carbon dioxide reduced this loss to a negligible amount. The tube *E*, filled with saturated brine, was provided for collecting any gaseous reaction products.

Analysis of the Liquid Reaction Products

Since the ether in the reaction products is mixed with unchanged alcohol, its quantitative estimation presents some difficulty. Extraction with solvents involves losses. The salting-out method used by Pease and Yung (9) is not quantitative when the ether content is less than one-third of the total liquid mixture.

The method of Kunke (6) was used with slight modifications. The ether vapor was entrained in a regulated current of air, the alcohol absorbed in 50% sulphuric acid contained in two gas washing bottles, and the ether oxidized quantitatively by acid dichromate in a Milligan bottle. The excess of dichromate was then titrated iodometrically. By careful manipulation of the process it was possible to check an analysis to within 0.1%.

Results

Curve *A*, Fig. 2, shows the different amounts of ether produced when catalysts of different water content are used. If the alumina contains more than 13.8% of water it has no effect on this reaction. If it contains 13.8% of water a trace of ether can be detected in the reaction products. As the amount of residual water in the catalyst is decreased, the activity increases until at a water content of about 5.5% the conversion to ether reaches a maximum. As the water content of the alumina is decreased further, the activity is lessened and falls steadily, becoming much lower when the catalyst is anhydrous.

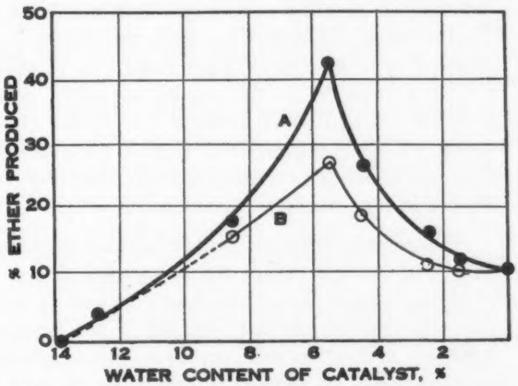

FIG. 2. *Graphical representation of the activity of alumina catalysts containing different amounts of residual water. A, originally; B, after the passage of 50 cc. of ethyl alcohol.*

Other workers have shown that the amount of catalyst used has a noticeable effect on the amount of ether produced, provided that the rate of flow is kept constant. It seems probable that this is simply an observation of the extent to which the catalyst has been poisoned. With a large amount of catalyst, 25 cc. of alcohol may be supplied before the activity is appreciably reduced by poisoning. In this study a comparatively small amount of catalyst (13 gm.) was used, and the decrease in activity is quickly noted. Curve *B*, Fig. 2 represents graphically the activity of the catalysts after the passage of 50 cc. of alcohol.

As will be seen from the curve, the lowering in efficiency is relatively greatest for the catalyst originally having the highest activity. The two curves converge, *i.e.*, the relative poisoning decreases until in the catalysts containing

no residual water, no difference in activity could be detected. The curves also appear to converge at zero activity (13.8% water). In no case was any gas produced.

Discussion

Though adsorptive capacity does not always parallel catalytic activity, nevertheless adsorption does play an important role. In catalysts of high water content, the forces causing adsorption are evidently saturated by the water already present. When activation brings these forces into play again, adsorption and catalysis can begin, although not necessarily at the same point of dehydration (8).

It has been suggested by several investigators that decrease in activity with time is due to auto-toxic adsorption of the reaction products. Thus Ipatiew (5) states that sorbed water slows down the catalytic dehydration of ether, and Engelder (2) has noted a similar effect in the dehydration of alcohol. This would explain why the most active catalysts are poisoned to the greatest extent. It does not, without amplification, explain why no decrease in activity was found with catalysts containing no residual water. Furthermore, Guichard has recently shown (4) that at 250° C., though the adsorption of alcohol is appreciable, *that of ether and water is practically zero.*

The maximum in the curves probably does not represent the point of greatest adsorption of alcohol, but rather that point at which the relative number and arrangement of alcohol molecules and alumina groups or atoms are most favorable for the reaction. Beyond this optimum, increased adsorption of alcohol would be unfavorable.

Adkins has contended that structural changes are brought about by heating and may sensibly affect the specificity of a given catalyst. In the present study no measurable amount of ethylene or hydrogen was produced. There was therefore no change induced in the course of the reaction. Either the different activation temperatures and resulting variation in residual water content cause no change in structure, or such a change does not affect the specificity of the catalyst.

An explanation for the foregoing experimental results has been suggested, but it awaits substantiation by further investigation.

References

1. Adkins, H. and Krause, A. C. J. Am. Chem. Soc. 44 : 385-392. 1922.
2. Engelder, C. J. J. Phys. Chem. 21 : 676-704. 1917.
3. Frazer, J. C. W. J. Phys. Chem. 34 : 2129-2179. 1930.
4. Guichard. Compt. rend. 198 : 573-575. 1934.
5. Ipatiew, W. Ber. 37 : 2986-3005. 1904.
6. Kunke, W. F. J. Assoc. Off. Agr. Chem. 16 : 348-361. 1933.
7. Munro, L. A. and Johnson, F. M. G. J. Ind. Eng. Chem. 17 : 88-92. 1925.
8. Munro, L. A. and McCubbin, J. W. Proc. Roy. Soc. Can. III, 27 : 29-33. 1934.
9. Pease, R. N. and Yung, C. C. J. Am. Chem Soc. 46 : 2397-2418. 1924.

NITROUS OXIDE AS AN OXIDIZING AGENT IN THE GASEOUS STATE[1]

By E. W. R. Steacie[2] and R. D. McDonald[3]

Abstract

The kinetics of a number of gaseous oxidation reactions have been investigated, using nitrous oxide as the oxidizing agent in place of oxygen. It is found that, in general, nitrous oxide is much less efficient as an oxidizing agent than is molecular oxygen. Nitrous oxide in most cases acts merely as a reservoir of atomic oxygen at temperatures where its rate of decomposition is appreciable.

Introduction

In view of the progress that is being made in the study of the kinetics of gaseous oxidations by molecular oxygen, it is of interest to extend the field of investigation to include similar reactions in which oxygen is replaced by other gases.

Dixon and Higgins (6) measured the temperatures of spontaneous ignition of jets of different combustible gases in an atmosphere of nitrous oxide, and found that these temperatures were usually slightly lower than those obtained with oxygen. Since such experiments involve great uncertainty as to gas composition, they cannot yield definite kinetic conclusions, but they seem to indicate that the mechanisms of the two oxidation processes are similar.

In the case of the hydrogen–nitrous oxide reaction, Hinshelwood (9) concluded that the rate of reaction was no greater than could be accounted for by the decomposition of nitrous oxide followed by rapid reaction of the oxygen and hydrogen. Melville (11) has recently carried out an extensive investigation of this reaction. His results showed that the reaction was from 90 to 500 times as fast as the nitrous oxide decomposition, depending on the experimental conditions. His results are satisfactorily explained by a chain mechanism, the chains being initiated by atomic oxygen formed by the decomposition of nitrous oxide.

It seems desirable to extend such investigations to other systems, and this paper deals with the oxidation of a number of substances by nitrous oxide.

I. Methyl Alcohol

The methyl alcohol–oxygen reaction has been investigated by Fort and Hinshelwood (7) This is a typical chain reaction, and it therefore seemed worth investigating the nitrous oxide–methyl alcohol reaction.

The apparatus was similar to that used in previous investigations (8, 14). The results obtained have already been described in detail (15). It was found that the reaction proceeded at a conveniently measurable rate at 500 to 570° C.

[1] *Manuscript received January 31, 1935.*
Contribution from the Physical Chemistry Laboratories, McGill University, Montreal, Quebec, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Holder of a fellowship under the National Research Council of Canada at the time when this work was performed.*

This is a much higher temperature than is required for the methyl alcohol–oxygen reaction. At such temperatures the observed rate is much faster than the rate of decomposition of nitrous oxide. It is therefore not possible to ascribe the reaction to a simple decomposition of nitrous oxide followed by oxidation of alcohol by the oxygen thus produced.

The decomposition of nitrous oxide is, however, appreciable in this temperature range. It therefore appears probable that the reaction is a chain process in which the chains are initiated by oxygen atoms derived from the nitrous oxide decomposition. The behavior is therefore similar to that found by Melville for the hydrogen–nitrous oxide reaction.

II. Ethylene

The ethylene–oxygen reaction proceeds at a measurable rate at 300 to 450° C. (3, 10, 16, 18). The nitrous oxide–ethylene reaction was found to be slow even at 530° C. At this temperature a large amount of tar and other condensable materials was formed. The main reaction seemed to be polymerization and decomposition of ethylene, followed by a slow oxidation of the products by nitrous oxide. On account of the fact that the non-volatile products formed reacted slowly with nitrous oxide, it would have been necessary to use a new reaction bulb for every run, and the investigation was therefore abandoned.

It was, however, definitely established that nitrous oxide was a very much less efficient oxidizing agent than oxygen, and that it is effective only at temperatures at which its decomposition is becoming appreciable.

III. Acetaldehyde

The acetaldehyde–oxygen reaction proceeds at quite low temperatures, *viz.*, 60 to 120° C. (1, 2, 8, 12).

About 40 runs were made with acetaldehyde–nitrous oxide mixtures. The reaction proceeded at a conveniently measurable rate at 450° C. It was accompanied by a pressure increase of about 115%. The reaction is complicated by the fact that at 450° C. the decomposition of acetaldehyde is fairly large, and depending on its partial pressure from 5 to 20% of the acetaldehyde disappearing does so by decomposition rather than oxidation.

The actual N_2O–CH_3CHO reaction is complex. It is slightly retarded by packing the reaction vessel, the rate diminishing about 20% when a 200 cc. silica bulb is filled with 1 in. lengths of $\frac{1}{8}$ in. tubing. The reaction is therefore a chain process. The products correspond in the main to oxidation to acetic acid followed by the decomposition of the acid to methane and carbon dioxide.

The kinetics of the reaction are complicated. For mixtures having the composition 1 CH_3CHO + 0.7, 1, 2, and 4 N_2O, the time to quarter-value passes through a minimum with increasing pressure for each mixture. For all mixtures T_{25} is between 10 and 15 min. at partial aldehyde pressures of about 5 cm. It decreases to a minimum of about four minutes at pressures between 7 and 10 cm., and then increases again. This results in rather complicated relations for the effects of the concentrations of the separate reactants.

Thus at low aldehyde pressures T_{25} is approximately inversely proportional to (N_2O), at intermediate pressures it is independent of it, and at high pressures it is proportional to it. At low nitrous oxide pressures T_{25} is inversely proportional to (CH_3CHO), while at higher pressures it is independent of (CH_3CHO).

On account of the complicated nature of the reaction, and the fact that the acetaldehyde decomposition causes serious errors in the results, it is not worth while reporting the experimental data in detail. There is, however, no doubt that nitrous oxide does not react with acetaldehyde at all at temperatures where the acetaldehyde–oxygen reaction would proceed practically instantaneously.

IV. Phosphine

The phosphine–oxygen reaction proceeds explosively at room temperature (within certain pressure limits) (4, 5, 19). With phosphine–nitrous oxide mixtures it was found that a 4 N_2O + 1 PH_3 mixture at 600° C. gave a pressure decrease of about 30% relative to the phosphine. Even at this high temperature, however, the reaction was fairly slow, T_{50} being about five minutes for total pressures of 20 cm. At 600° C. and total pressures of about 32 cm. explosions occurred. It is apparent that here again preliminary dissociation of nitrous oxide is necessary.

V. Carbon Disulphide

The carbon disulphide–oxygen reaction also possesses critical pressure limits, and within these explosions occur at temperatures as low as 140° C. (13, 17). The reaction between carbon disulphide and nitrous oxide is negligibly slow at temperatures below 600° C.

Discussion

As mentioned above, Dixon and Higgins found uniformly lower ignition temperatures in nitrous oxide than in oxygen. The variety of reactions which we have investigated show, however, that nitrous oxide is a very inefficient oxidizing agent compared with oxygen. In general, oxidation by means of nitrous oxide occurs only at temperatures at which its dissociation is becoming appreciable. This result, however, is not incompatible with the work of Dixon and Higgins. The substances they investigated all had fairly high ignition temperatures. At such temperatures the decomposition of nitrous oxide is rapid, and the oxidation is really occurring through atomic oxygen.

It may therefore be concluded that, in general, nitrous oxide does not itself act as an oxidizing agent in the gaseous state. It functions merely as a reservoir of atomic oxygen when the temperature is sufficiently high.

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References

1. Bodenstein, M. Sitzb. preuss. Akad. Wiss. 73-88. 1931.
2. Bodenstein, M. Z. physik. Chem. Abt. B 12 : 151-164. 1931.
3. Bone, W. A., Haffner, A. E. and Rance, H. F. Proc. Roy. Soc. Lond. A 143 : 16-37. 1933.
4. Dalton, R. H. Proc. Roy. Soc. Lond. A 128 : 263-275. 1930.
5. Dalton, R. H. and Hinshelwood, C. N. Proc. Roy. Soc. Lond. A 125 : 294-308. 1929.
6. Dixon, H. B. and Higgins, W. F. Mem. Proc. Manchester Lit. Phil. Soc. 71 : 15-22. 1926–27.
7. Fort, R. and Hinshelwood, C. N. Proc. Roy. Soc. Lond. A 129 : 284-299. 1930.
8. Hatcher, W. H., Steacie, E. W. R. and Howland, F. Can. J. Research, 7 : 149-161. 1932.
9. Hinshelwood, C. N. Proc. Roy. Soc. Lond. A 106 : 292-298. 1924.
10. Lenher, S. J. Am. Chem. Soc. 53 : 3737-3751; 3752-3765. 1931.
11. Melville, H. W. Proc. Roy. Soc. Lond. A 142 : 524-545. 1933.
12. Pease, R. N. J. Am. Chem. Soc. 55 : 2753-2761. 1933.
13. Ritchie, A., Brown, R. R. H. and Muir, J. J. Proc. Roy. Soc. Lond. A 137 : 511-519. 1932.
14. Steacie, E. W. R. Proc. Roy. Soc. Lond. A 127 : 314-330. 1930.
15. Steacie, E. W. R. and McDonald, R. D. J. Phys. Chem. 38 : 1031-1043. 1934.
16. Steacie, E. W. R. and Plewes, A. C. Proc. Roy. Soc. Lond. A 146 : 72-82. 1934.
17. Thompson, H. W. Z. physik. Chem. Abt. B 10 : 273-295. 1930
18. Thompson, W. H. and Hinshelwood, C. N. Proc. Roy. Soc. Lond. A 125 : 277-291. 1929.
19. Trautz, M. and Gabler, W. Z. anorg. allgem. Chem. 180 : 321-354. 1929.